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(54) Title: AL-2 NEUROTROPHIC FACTOR

(57) Abstract

The present invention provides nucleic acids encoding AL-2 protein, as well as AL-2 protein produced by recombinant DNA methods. Such AL-2 protein and nucleic acid are useful in preparing antibodies and antagonists and in diagnosing and treating various neuronal disorders and disorders or conditions associated with angiogenesis.

# AL-2 NEUROTROPHIC FACTOR

## INTRODUCTION

### Technical Field

This application relates to a receptor protein tyrosine kinase ligand and its uses. In particular this application relates to the production and use of purified forms of AL-2 and related proteins.

### Background

Protein neurotrophic factors, or neurotrophins, which influence growth and development of the vertebrate nervous system, are believed to play an important role in promoting the differentiation, survival, and function of diverse groups of neurons in the brain and periphery. Neurotrophic factors are believed to have important signaling functions in neural tissues, based in part upon the precedent established with nerve growth factor (NGF). NGF supports the survival of sympathetic, sensory, and basal forebrain neurons both *in vitro* and *in vivo*. Administration of exogenous NGF rescues neurons from cell death during development. Conversely, removal or sequestration of endogenous NGF by administration of anti-NGF antibodies promotes such cell death (Heumann, *J. Exp. Biol.*, 132:133-150 (1987); Hefti, *J. Neurosci.*, 6:2155-2162 (1986); Thoenen *et al.*, *Annu. Rev. Physiol.*, 60:284-335 (1980)).

Additional neurotrophic factors related to NGF have since been identified. These include brain-derived neurotrophic factor (BDNF) (Leibrock, *et al.*, *Nature*, 341:149-152 (1989)), neurotrophin-3 (NT-3) (Kaisho, *et al.*, *FEBS Lett.*, 266:187 (1990); Maisonpierre, *et al.*, *Science*, 247:1446 (1990); Rosenthal, *et al.*, *Neuron*, 4:767 (1990)), and neurotrophin 4/5 (NT-4/5) (Berkmeier, *et al.*, *Neuron*, 7:857-866 (1991)).

Neurotrophins, similar to other polypeptide growth factors, affect their target cells through interactions with cell surface receptors. According to our current understanding, two kinds of transmembrane glycoproteins act as receptors for the known neurotrophins. Equilibrium binding studies have shown that neurotrophin-responsive neuronal cells possess a common low molecular weight (65,000 - 80,000 Daltons), a low affinity receptor typically referred to as $p75^{LNGFR}$ or p75, and a high molecular weight (130,000-150,000 Dalton) receptor. The high and low affinity receptors are members of the trk family of receptor tyrosine kinases.

Receptor tyrosine kinases are known to serve as receptors for a variety of protein factors that promote cellular proliferation, differentiation, and survival. In addition to the trk receptors, examples of other receptor tyrosine kinases include the receptors for epidermal growth factor (EGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF). Typically, these receptors span the cell membrane, with one portion of the receptor being intracellular and in contact with the cytoplasm, and another portion of the receptor being extracellular. Binding of a ligand to the extracellular portion of the receptor induces tyrosine kinase activity in the intracellular portion of the receptor, with ensuing phosphorylation of various intracellular proteins involved in cellular signaling pathways.

Recently, a receptor tyrosine kinase subclass referred to as the Eph receptor subclass or family has been identified. Eph was the first member of this Eph subclass of receptor tyrosine kinases to be identified and characterized by molecular cloning (Hirai *et al.*, *Science*, 238:1717-1720 (1987)). The name Eph is derived from the name of the cell line from which the Eph cDNA was first isolated, the erythropoietin-producing human hepatocellular carcinoma cell line, ETL-1. The general structure of Eph is similar to that of other receptor tyrosine kinases and consists of an extracellular domain, a single membrane spanning region, and a conserved

or rearrangements in, for example, hemopoietic tumors and lymphoid tumor cell lines. Over-expression of Myk-1 (a murine homolog of human Htk (Bennett *et al., J. Biol. Chem.*, 269(19):14211-8 (1994)) was found in the undifferentiated and invasive mammary tumors of transgenic mice expressing the Ha-ras oncogene. (Andres *et al.,Oncogene*, 9(5):1461-7 (1994) and Andres *et al.,Oncogene*, 9(8):2431 (1994)).

In addition to their roles in carcinogenesis, a number of transmembrane tyrosine kinases have been reported to play key roles during development. Some receptor tyrosine kinases are developmentally regulated and predominantly expressed in embryonic tissues. Examples include Cek1, which belongs to the FGF subclass, and the Cek4 and Cek5 tyrosine kinases (Pasquale *et al., Proc. Natl. Acad. Sci., USA*, 86:5449-5453 (1989); Sajjadi *et al., New Biol.*, 3(8):769-78 (1991); and Pasquale, *Cell Regulation*, 2:523-534 (1991)).

Eph family members are expressed in many different adult tissues, with several family members expressed in the nervous system or specifically in neurons (Maisonpierre *et al., Oncogene*, 8:3277-3288 (1993); Lai *et al., Neuron*, 6:691-704 (1991)).

The aberrant expression or uncontrolled regulation of any one of these receptor tyrosine kinases can result in different malignancies and pathological disorders. Therefore, there exists a need to identify means to regulate, control and manipulate receptor tyrosine kinases and their ligands in order to provide new and additional means for the diagnosis and therapy of Eph-pathway related disorders and cellular processes. The present application provides the clinician and researcher with such means by providing new molecules that are specific for interacting with Eph-family receptors. These compounds and their methods of use, as provided herein, allow exquisite therapeutic control and specificity. Additional advantages are provided as well.

## SUMMARY

The present invention provides a novel cytokine, an Eph-related tyrosine kinase receptor ligand referred to as AL-2.

The present invention provides nucleic acid encoding AL-2, particularly two forms referred to herein as AL-2s ("AL-2-short") and AL-2l ("AL-2-long"), and methods to use the nucleic acid to produce AL-2 in recombinant host cells for diagnostic or therapeutic purposes. Also provided are uses of nucleic acids encoding AL-2, and portions thereof, to identify related nucleic acids in the cells or tissues of various animal species.

By providing the full nucleotide coding sequence for AL-2, the invention enables the production of AL-2 by means of recombinant DNA technology, thereby making available for the first time sufficient quantities of substantially pure AL-2 protein or AL-2 antagonists for diagnostic and therapeutic uses. For example, method embodiments include treatment or prevention of a variety of neurological disorders and diseases as well as conditions that are angiogenesis-dependent such as solid tumors, diabetic retinopathy, rheumatoid arthritis, and wound healing.

Also provided are derivatives and modified forms of AL-2, including amino acid sequence variants and covalent derivatives thereof, as well as antagonists of AL-2, that are preferably biologically active (*e.g.*, antigenically active. In one embodiment, the invention provides a soluble form of the ligand with at least the transmembrane region deleted. Usually, the cytoplasmic domain will also be absent. Immunogens are provided for raising antibodies, as well as to obtain antibodies, capable of binding to, preferably neutralizing, AL-2 or derivatives or modified forms thereof.

In a preferred embodiment, the invention provides AL-2 that is free of other human proteins.

initiation methionine. The C-terminal hydrophobic transmembrane domain extends from amino acid Leu-220 to Ala-245. The deduced extracellular domain sequence includes amino acids Gly-27 to Pro-219.

Figure 3A-3B depicts an alignment of the AL-2l nucleotide sequence with human EST sequence H10006 (SEQ ID NO: 5).

Figure 4 shows a comparison of the AL-2l and AL-2s amino acid sequences with that of Lerk2 (Beckmann *et al., EMBO J.*, 13:3757-3762 (1994)) and human Htk-L (Bennett *et al., Proc. Natl. Acad. Sci. USA*, 92:1866-70 (1995); WO 96/02645 published February 1, 1996; both are incorporated by reference herein). Identical amino acids are boxed, and gaps introduced for optimal alignment are indicated by dashes. Conserved cysteine residues can be seen. The deduced C-terminal amino acid for AL-2s is valine.

Figure 5 shows a comparison of the AL-2l amino acid sequences with that of Lerk2 and human Htk-L. Identical amino acids are boxed, and gaps introduced for optimal alignment are indicated by dashes. Conserved cysteine residues can be seen.

## DETAILED DESCRIPTION

"AL-2" or "AL-2 protein" refers to a polypeptide or protein encoded by the AL-2 nucleotide sequence set forth in Figures 1A-1B (showing AL-2l) or 2 (showing AL-2s); a polypeptide that is the translated amino acid sequence set forth in Figures 1A-1B or 2A-2B; fragments thereof having greater than about 5 contiguous amino acid residues and comprising an immune epitope or other biologically active site of AL-2; amino acid sequence variants of the amino acid sequence set forth in Figures 1A-1B or 2A-2B wherein one or more amino acid residues are added at the N- or C-terminus of, or within, said Figures 1A-1B or 2A-2B sequences or its fragments as defined above; amino acid sequence variants of said Figures 1A-1B or 2A-2B sequences or its fragments as defined above wherein one or more amino acid residues of said Figures 1A-2B or 2A-2B sequences or fragment thereof are deleted, and optionally substituted by one or more amino acid residues; and derivatives of the above proteins, polypeptides, or fragments thereof, wherein an amino acid residue has been covalently modified so that the resulting product is a non-naturally occurring amino acid. Preferred embodiments retain a biological property of AL-2. AL-2 amino acid sequence variants may be made synthetically, for example, by site-directed or PCR mutagenesis, or may exist naturally, as in the case of allelic forms and other naturally occurring variants of the translated amino acid sequence set forth in Figures 1A-1B or 2A-2B that occur in human or other animal species. Accordingly, within the scope of the present invention are AL-2 proteins derived from other animal species, preferably mammalian, including but not limited to murine, rat, bovine, porcine, or various primates. As used herein, the term "AL-2" includes membrane-bound proteins (comprising a cytoplasmic domain, a transmembrane region, and an extracellular domain), including the long and short forms of AL-2, as well as truncated proteins that retain Eph-family-receptor binding property. Truncated AL-2 proteins include, for example, soluble AL-2 comprising only the extracellular (receptor binding) domain. Such fragments, variants, and derivatives exclude any polypeptide heretofore identified, including any known neurotrophic factor, such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), Eph family receptor ligand such as Erk-L or Lerk-2, as well as statutorily obvious variants thereof. A preferred AL-2 is one having a contiguous amino acid sequence of or derived from mature AL-2 shown in Figures 1A-1B or 2A-2B.

residues). The preferred AL-2 is human AL-2, especially native human AL-2 having the sequence shown in Figures 1A-1B or 2A-2B.

One embodiment of the present invention provides soluble AL-2. By "soluble AL-2" is meant AL-2 which is essentially free of at least a transmembrane sequence and, optionally, the intracellular domain of native AL-2. By "essentially free" is meant that the soluble AL-2 sequence has less than 2% of the transmembrane domain, preferably less than 1% of the transmembrane domain, and more preferably less than 0.5% of this domain. The transmembrane domain of the native human mature amino acid sequences are delineated in Figures 1A-1B and 2A-2B (for AL-2l and AL-2s, respectively), *i.e.*, resides Gly-27 to Pro-219. Soluble AL-2s have therapeutic advantages because they are generally soluble in the patient's blood stream. Similarly, soluble ligands may prove to be particularly useful as diagnostics since they are expected to have a reduced tendency to incorporate in the cell membrane. Soluble AL-2 polypeptides comprise all or part of the extracellular domain of a native AL-2 but lack the transmembrane region that would cause retention of the polypeptide on a cell membrane. Soluble AL-2 polypeptides advantageously comprise the native (or a heterologous) signal peptide when initially synthesized to promote secretion, but the signal peptide is cleaved upon secretion. In preferred embodiments, the soluble AL-2 polypeptides retain the ability to bind an Eph-family receptor with preferences as discussed herein. Soluble AL-2 can also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble AL-2 protein is capable of being secreted or otherwise isolated.

In one embodiment a soluble AL-2 is an "immunoadhesin". The term "immunoadhesin" is used interchangeably with the expression "AL-2-immunoglobulin chimera" and refers to a chimeric molecule that combines the extracellular domain ("ECD") of AL-2 with an immunoglobulin sequence. The immunoglobulin sequence preferably, but not necessarily, is an immunoglobulin constant domain. The immunoglobulin moiety in the chimeras of the present invention may be obtained from IgG-1, IgG-2, IgG-3 or IgG-4 subtypes, IgA, IgE, IgD or IgM, but preferably IgG-1 or IgG-3. The expression "extracellular domain" or "ECD" when used herein refers to any polypeptide sequence that shares a receptor binding function of the extracellular domain of the naturally occurring AL-2 disclosed herein. Receptor binding function refers to the ability of the polypeptide to bind the extracellular domain of a Eph-family receptor, with preferences as discussed herein, and, optionally, activate the receptor. Accordingly, it is not necessary to include the entire extracellular domain since smaller segments are commonly found to be adequate for receptor binding. The term ECD encompasses polypeptide sequences in which the cytoplasmic domain and hydrophobic transmembrane sequence (and, optionally, 1-20 amino acids amino-terminal to the transmembrane domain) of the mature AL-2 have been deleted. The extracellular domain sequence of AL-2 is provided in Figures 1A-1B and 2A-2B.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising the entire AL-2, or a portion thereof, fused to a "tag polypeptide." The tag polypeptide has sufficient amino acids to provide an antibody-binding epitope but not interfere with activity of the AL-2. The tag polypeptide preferably also is fairly unique so that an antibody against it does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least 6 amino acid residues and usually between about 8-50 amino acid residues, preferably between about 9-30 residues.

90 bases. For fragments, the percent identity is calculated for that portion of a native sequence that is present in the fragment.

In one embodiment an isolated AL-2 protein induces phosphorylation of an Eph-family receptor and contains an amino acid sequence selected from the group consisting of (a) the amino acid sequence for mature AL-2l, (b) the amino acid sequence for mature AL-2s, (c) the naturally occurring amino acid sequence for mature AL-2 from a non-human animal species, (d) allelic variants of the sequences of (a), (b), or (c), and (e) the sequences of (a), (b), (c), or (d) having a single preferred conservative amino acid substitution as defined in Table 1. In a preferred embodiment the phosphorylation-inducing AL-2 has the amino acid sequence for mature human AL-2 shown in Figures 1A-1B or 2A-2B. Generally the AL-2 will be a chimera, membrane or liposome bound, or epitope tagged and "clustered" (see WO 95/27060, which is incorporated herein by reference), thus mimicking its membrane-bound state and ability to induce receptor phosphorylation. In another embodiment an isolated AL-2 protein binds to the Eph-family receptor and contains an amino acid sequence selected from the group consisting of (a) the amino acid sequence for mature AL-2l, (b) the amino acid sequence for mature AL-2s, (c) the naturally occurring amino acid sequence for mature AL-2 from a non-human animal species, (d) allelic variants of the sequences of (a), (b), or (c), and (e) the sequences of (a), (b), (c), or (d) having a single preferred conservative amino acid substitution as defined in Table 1. In a preferred embodiment the AL-2 has the amino acid sequence for mature human AL-2 shown in Figures 1A-1B or 2A-2B. In another embodiment isolated soluble AL-2 binds to a Eph-family receptor and contains an amino acid sequence selected from the group consisting of (a) the amino acid sequence for mature soluble AL-2l, (b) the amino acid sequence for mature soluble AL-2s, (c) the naturally occurring amino acid sequence for mature soluble AL-2 from a non-human animal species, (d) allelic variants of the sequences of (a), (b), or (c), and (e) the sequences of (a), (b), (c), or (d) having a single preferred conservative amino acid substitution as defined in Table 1. In a preferred embodiment the soluble AL-2 has the amino acid sequence for mature soluble human AL-2 shown in Figures 1A-1B or 2A-2B. In another preferred embodiment, the soluble AL-2 is a chimeric polypeptide containing an amino acid sequence encoding mature soluble AL-2 fused to an immunoglobulin sequence. In a more preferred embodiment the chimeric polypeptide contains a fusion of an AL-2 extracellular domain sequence to an immunoglobulin constant domain sequence. Preferably the constant domain sequence is that of an immunoglobulin heavy chain. Also preferred are chimeric polypeptides containing a mature, soluble Al-2 amino acid sequence fused to an epitope tag polypeptide sequence.

AL-2 can be recovered from culture of cells expressing AL-2, preferably from the culture medium as a secreted polypeptide; although, AL-2 can be recovered from host cell lysates when directly produced without a secretory signal. When AL-2 is membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.*, Triton-X 100™). When AL-2 is produced in a recombinant cell other than one of human origin, AL-2 is completely free of proteins or polypeptides of human origin. However, it is necessary to purify AL-2 from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous in AL-2. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. Then AL-2 is purified from contaminant soluble proteins and polypeptides, with the following procedures being exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-

One useful approach is called "alanine scanning mutagenesis." Here, a an amino acid residue or group of target residues are identified (*e.g.*, charged residues such as arginine, aspartic acid, histidine, lysine, and glutamic acid) and, by means of recombinant DNA technology, replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. (Cunningham, *et al.*, Science, 244:1081-1085 (1989)). Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at or for the sites of substitution. Obviously, such variations that, for example, convert the amino acid sequence set forth in Figures 1A-1B and 2A-2B to the amino acid sequence of a known neurotrophic factor, such as NGF, BDNF, NT-3, NT-4/5, Eph-family receptor ligand (*e.g.*, see Figures 4 and 5), or another known polypeptide or protein are not included within the scope of this invention, nor are any other fragments, variants, and derivatives of the amino acid AL-2 that are not novel and unobvious over the prior art. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to optimize the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed AL-2 variants are screened for functional activity.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably about 1 to 10 residues, and typically are contiguous. Deletions from regions of substantial homology with other tyrosine kinase receptor ligands, for example, are more likely to affect the functional activity of AL-2. Generally, the number of consecutive deletions will be selected so as to preserve the tertiary structure of AL-2 in the affected domain, *e.g.*, beta-pleated sheet or alpha helix.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one amino acid residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions, *i.e.*, insertions made within the amino acid sequence set forth in Figures 1A-1B or 2A-2B, may range generally from about 1 to 10 residues, more preferably 1 to 5, even more preferably 1 to 3, and most preferably 1 to 2. Examples of terminal insertions include AL-2 with an N-terminal methionyl residue (such as may result from the direct expression of AL-2 in recombinant cell culture), and AL-2 with a heterologous N-terminal signal sequence to improve the secretion of AL-2 from recombinant host cells. Such signal sequences generally will be homologous to the host cell used for expression of AL-2, and include STII or lpp for *E. coli*, alpha factor for yeast, and viral signals such as herpes gD for mammalian cells. Other insertions include the fusion to the N- or C-terminus of AL-2 of immunogenic polypeptides (for example, bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli* trp locus, or yeast protein), and C-terminal fusions with proteins having a long half-life such as immunoglobulin constant regions, albumin, or ferritin, as described in PCT Pat. Pub. No. WO 89/02922 published April 6, 1989.

The third group of variants are those in which at least one amino acid residue in the amino acid sequence set forth in Figures 1A-1B or 2A-2B, preferably one to four, more preferably one to three, even more preferably one to two, and most preferably only one, has been removed and a different residue inserted in its place. The sites of greatest interest for making such substitutions are in the regions of the amino acid sequence set forth in Figures 1A-1B or 2A-2B that have the greatest homology with other tyrosine kinase receptor ligands (for non-limiting examples, see comparisons in Figures 4 and 5). Those sites are likely to be important to the

of AL-2 for functional activity may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking.

Additional sites for mutation are those sites that are conserved in AL-2 amongst species variants of AL-2 but are not conserved between AL-2 and another ligand in the Eph ligand family, preferably between AL-2 and at least two ligands, and more preferably at least three ligands. Such sites, which are not conserved between AL-2 and another transmembrane-ligand, are candidates sites for modulating receptor specificity and selectivity. Sites that are conserved between AL-2 and other transmembrane-ligands are candidate sites for modulating activities shared by transmembrane-ligands, such as stability, folding, tertiary conformation, protease susceptibility, and amount of ligand specific activity.

A comparison of AL-2 amino acid sequences with other Eph-family receptor ligand sequences (see Figures 4 and 5) reveals AL-2 as a new Eph-family receptor ligand. AL-2, having a transmembrane sequence, is more closely related to other transmembrane-containing ligands than to the GPI-anchored ligands, of which AL-1 is an example. Transmembrane-containing ligands include Lerk-2, a ligand for the Eph-related receptor Hek5, and Htk-L, a ligand for the Htk receptor. Percent identities of ligand comparisons are provided in Table 2, in which "ECD" indicates extracellular domain.

**TABLE 2**

**% IDENTITY**

| Ligand | Full Length | ECD | Cytoplasmic Domain |
|---|---|---|---|
| Lerk2 vs. HtkL | 56.0% | 49.3% | 74.7% |
| AL-2 vs Lerk2 | 41.5% | 42.1% | 48.2% |
| AL-2 vs HtkL | 40.8% | 39.5% | 56.6% |
| AL-2 vs AL-1 | 28.0% | | |

Covalent modifications of AL-2 molecules also are included within the scope of this invention. For example, covalent modifications are introduced into AL-2 by reacting targeted amino acid residues of the AL-2 with an organic derivatizing agent that is capable of reacting with selected amino acid side chains or the N- or C-terminal residues.

Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α-amino-containing residues include imidoesters such as methyl picolinimidate;

subject cell and cultures derived therefrom without regard for the number of times the cultures have been passaged. It should also be understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations.

"Plasmids" are DNA molecules that are capable of replicating within a host cell, either extrachromosomally or as part of the host cell chromosome(s), and are designated by a lower case "p" preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are commercially available, are publicly available on an unrestricted basis, or can be constructed from such available plasmids as disclosed herein and/or in accordance with published procedures. In certain instances, as will be apparent to the ordinarily skilled artisan, other plasmids known in the art may be used interchangeably with plasmids described herein.

"Control sequences" refers to DNA sequences necessary for the expression of an operably linked nucleotide coding sequence in a particular host cell. The control sequences that are suitable for expression in prokaryotes, for example, include origins of replication, promoters, ribosome binding sites, and transcription termination sites. The control sequences that are suitable for expression in eukaryotes, for example, include origins of replication, promoters, ribosome binding sites, polyadenylation signals, and enhancers.

An "exogenous" element is one that is foreign to the host cell, or homologous to the host cell but in a position within the host cell in which the element is ordinarily not found.

"Digestion" of DNA refers to the catalytic cleavage of DNA with an enzyme that acts only at certain locations in the DNA. Such enzymes are called restriction enzymes or restriction endonucleases, and the sites within DNA where such enzymes cleave are called restriction sites. If there are multiple restriction sites within the DNA, digestion will produce two or more linearized DNA fragments (restriction fragments). The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors, and other requirements as established by the enzyme manufacturers are used. Restriction enzymes commonly are designated by abbreviations composed of a capital letter followed by other letters representing the microorganism from which each restriction enzyme originally was obtained and then a number designating the particular enzyme. In general, about 1 µg of DNA is digested with about 1-2 units of enzyme in about 20 µl of buffer solution. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer, and/or are well known in the art.

"Recovery" or "isolation" of a given fragment of DNA from a restriction digest typically is accomplished by separating the digestion products, which are referred to as "restriction fragments," on a polyacrylamide or agarose gel by electrophoresis, identifying the fragment of interest on the basis of its mobility relative to that of marker DNA fragments of known molecular weight, excising the portion of the gel that contains the desired fragment, and separating the DNA from the gel, for example by electroelution.

"Ligation" refers to the process of forming phosphodiester bonds between two double-stranded DNA fragments. Unless otherwise specified, ligation is accomplished using known buffers and conditions with 10 units of T4 DNA ligase per 0.5 µg of approximately equimolar amounts of the DNA fragments to be ligated.

"Oligonucleotides" are short-length, single- or double-stranded polydeoxynucleotides that are chemically synthesized by known methods (involving, for example, triester, phosphoramidite, or phosphonate chemistry), such as described by Engels, *et al.*, *Agnew. Chem. Int. Ed. Engl.*, 28:716-734 (1989). They are then purified, for example, by polyacrylamide gel electrophoresis.

several DNAs and covalently joined to one another to construct the entire coding sequence. The preferred means of covalently joining DNA fragments is by ligation using a DNA ligase enzyme, such as T4 DNA ligase.

"Isolated" AL-2 nucleic acid is AL-2 nucleic acid that is identified and separated from (or otherwise substantially free from) contaminant nucleic acid encoding another polypeptide or from nucleic acid with which it is normally associated in the natural source of AL-2 nucleic acid. Isolated AL-2 nucleic acid molecules therefore are distinguished from the AL-2 nucleic acid molecule as it occurs naturally in cells. However, an isolated AL-2 nucleic acid molecule includes AL-2 nucleic acid molecules contained in cells that ordinarily express AL-2 where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells. The isolated AL-2 nucleic acid can be incorporated into a plasmid or expression vector for *in vitro*, *ex vivo* or *in vivo* use, or can be labeled for diagnostic and probe purposes, using a label as described further herein in the discussion of diagnostic assays and nucleic acid hybridization methods.

For example, isolated AL-2 DNA, or a fragment thereof comprising at least about 15 nucleotides, is used as a hybridization probe to detect, diagnose, or monitor disorders or diseases that involve changes in AL-2 expression, such as may result from neuron damage. In one embodiment of the invention, total RNA in a tissue sample from a patient (that is, a human or other mammal) can be assayed for the presence of AL-2 messenger RNA, wherein the decrease in the amount of AL-2 messenger RNA is indicative of neuronal degeneration.

The present invention further provides antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target AL-2 mRNA (sense) or AL-2 DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of AL-2 cDNA. Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to create an antisense or a sense oligonucleotide, based upon a cDNA sequence for a given protein is described for example, in Stein *et al.*, *Cancer Res.*, 48:2659 (1988) and van der Krol *et al.*, *BioTechniques*, 6:958, 1988. Although not to be restricted by the following working model, it is generally believed that binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block translation (RNA) or transcription (DNA) by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense oligonucleotides can be used to block expression of AL-2 proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo (i.e.*, capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences. Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increase affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes can be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, CaPO4-mediated DNA transfection,

well known (Keller *et al.*, *DNA Probes*, pp.11-18 (Stockton Press, 1989)). Typically, the hybridization probe or primer will contain 10-25 or more nucleotides, and will include at least 5 nucleotides on either side of the sequence encoding the desired mutation so as to ensure that the oligonucleotide will hybridize preferentially to the single-stranded DNA template molecule.

Multiple mutations are introduced into AL-2 DNA to produce amino acid sequence variants of AL-2 comprising several or a combination of insertions, deletions, or substitutions of amino acid residues as compared to the amino acid sequence set forth in Figures 1A-1B or 2A-2B. If the sites to be mutated are located close together, the mutations may be introduced simultaneously using a single oligonucleotide that encodes all of the desired mutations. If, however, the sites to be mutated are located some distance from each other (separated by more than about ten nucleotides), it is more difficult to generate a single oligonucleotide that encodes all of the desired changes. Instead, one of two alternative methods may be employed.

In the first method, a separate oligonucleotide is generated for each desired mutation. The oligonucleotides are then annealed to the single-stranded template DNA simultaneously, and the second strand of DNA that is synthesized from the template will encode all of the desired amino acid substitutions.

The alternative method involves two or more rounds of mutagenesis to produce the desired mutant. The first round is as described for introducing a single mutation: a single strand of a previously prepared AL-2 DNA is used as a template, an oligonucleotide encoding the first desired mutation is annealed to this template, and a heteroduplex DNA molecule is then generated. The second round of mutagenesis utilizes the mutated DNA produced in the first round of mutagenesis as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

PCR mutagenesis is also suitable for making amino acid sequence variants of AL-2 (Higuchi, in *PCR Protocols*, pp.177-183 (Academic Press, 1990); Vallette *et al.*, *Nuc. Acids Res.*, 17:723-733 (1989)). Briefly, when small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in a template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template. For introduction of a mutation into a plasmid DNA, for example, one of the primers is designed to overlap the position of the mutation and to contain the mutation; the sequence of the other primer must be identical to a nucleotide sequence within the opposite strand of the plasmid DNA, but this sequence can be located anywhere along the plasmid DNA. It is preferred, however, that the sequence of the second primer is located within 200 nucleotides from that of the first, such that in the end the entire amplified region of DNA bounded by the primers can be easily sequenced. PCR amplification using a primer pair like the one just described results in a population of DNA fragments that differ at the position of the mutation specified by the primer, and possibly at other positions, as template copying is somewhat error-prone (Wagner *et al.*, in *PCR Topics*, pp.69-71 (Springer-Verlag, 1991)).

If the ratio of template to product amplified DNA is extremely low, the majority of product DNA fragments incorporate the desired mutation(s). This product DNA is used to replace the corresponding region in the plasmid that served as PCR template using standard recombinant DNA methods. Mutations at separate

The simplest and most straightforward immunoadhesin design combined the binding region(s) of the 'adhesin' protein (in this case AL-2) with the hinge and Fc regions of an immunoglobulin heavy chain. Ordinarily, when preparing chimeras of the present invention, nucleic acid encoding the extracellular domain or a fragment thereof of AL-2 will be fused C-terminally to nucleic acid encoding the N-terminus of an immunoglobulin constant domain sequence, however N-terminal fusions are also possible. Typically, in such fusions the encoded chimeric polypeptide will retain at least functionally active hinge, CH2 and CH3 domains of the constant region of an immunoglobulin heavy chain. Fusions are also made to the C-terminus of the Fc portion of a constant domain, or immediately N-terminal to the CH1 of the heavy chain or the corresponding region of the light chain. The precise site at which the fusion is made is not critical; particular sites are well known and may be selected in order to optimize the biological activity, secretion or binding characteristics of AL-2-immunoglobulin chimeras.

In some embodiments, chimeras are assembled as monomers, or hetero- or homo-multimers, and particularly as dimers or tetramers, essentially as illustrated in WO 91/08298. In a preferred embodiment, the AL-2 extracellular domain sequence is fused to the N-terminus of the C-terminal portion of an antibody (in particular the Fc domain), containing the effector functions of an immunoglobulin, *e.g.*, immunoglobulin $G_1$ (IgG-1). It is possible to fuse the entire heavy chain constant region to the AL-2 extracellular domain sequence. Preferably a sequence beginning in the hinge region just upstream of the papain cleavage site (which defines IgG Fc chemically; residue 216, taking the first residue of heavy chain constant region to be 114, or analogous sites of other immunoglobulins) is used in the fusion. In one embodiment, an AL-2 amino acid sequence is fused to the hinge region and CH2 and CH3 or CH1, hinge, CH2 and CH3 domains of an IgG-1, IgG-2, or IgG-3 heavy chain. The precise site at which the fusion is made is not critical, and the optimal site can be determined by routine experimentation. The immunoglobulin portion can genetically engineered or chemically modified to inactivate a biological activity of the immunoglobulin portion, such as T-cell binding, while retaining desirable properties such as its scaffolding property for presenting AL-2 function to an axon or target cell. Chimeras can be assembled as multimers, particularly as homo-dimers or -tetramers. Generally, these assembled immunoglobulins will have known unit structures. A basic four chain structural unit is the form in which IgG, IgD, and IgE exist. A four unit is repeated in the higher molecular weight immunoglobulins; IgM generally exists as a pentamer of basic four units held together by disulfide bonds. IgA globulin, and occasionally IgG globulin, may also exist in multimeric form in serum. In the case of multimer, each four unit may be the same or different. Alternatively, the AL-2 extracellular domain sequences can be inserted between immunoglobulin heavy chain and light chain sequences such that an immunoglobulin comprising a chimeric heavy chain is obtained. In this embodiment, the sequences are fused to the 3' end of an immunoglobulin heavy chain in each arm of an immunoglobulin, either between the hinge and the CH2 domain, or between the CH2 and CH3 domains (see Hoogenboom *et al.*, *Mol. Immunol.*, 28:1027-1037 (1991)). The presence of an immunoglobulin light chain is not required in the immunoadhesins of the present invention; an immunoglobulin light chain might be present either covalently associated to a immunoglobulin heavy chain fusion polypeptide, or directly fused to the AL-2 extracellular domain. In the former case, DNA encoding an immunoglobulin light chain is typically coexpressed with the DNA encoding the AL-2-immunoglobulin heavy chain fusion protein. Upon secretion, the hybrid heavy chain and the light chain will be covalently associated to provide an immunoglobulin-like structure comprising

(PCR) techniques. The cDNAs encoding the 'adhesin' and the Ig parts of the immunoadhesin are inserted in tandem into a plasmid vector that directs efficient expression in the chosen host cells. For expression in mammalian cells pRK5-based vectors (Schall *et al.*, *Cell.* 61:361-370 (1990)) and CDM8-based vectors (Seed, *Nature.* 329:840 (1989)). The exact junction can be created by removing the extra sequences between the designed junction codons using oligonucleotide-directed deletional mutagenesis (Zoller *et al.*, *Nucleic Acids Res.*, 10:6487 (1982); Capon *et al.*, *Nature.* 337:525-531 (1989)). Synthetic oligonucleotides can be used, in which each half is complementary to the sequence on either side of the desired junction; ideally, these are 36 to 48-mers. Alternatively, PCR techniques can be used to join the two parts of the molecule in-frame with an appropriate vector.

The choice of host cell line for the expression of AL-2-Ig immunoadhesins depends mainly on the expression vector. Another consideration is the amount of protein that is required. Milligram quantities often can be produced by transient transfections. For example, the adenovirus EIA-transformed 293 human embryonic kidney cell line can be transfected transiently with pRK5-based vectors by a modification of the calcium phosphate method to allow efficient immunoadhesin expression. CDM8-based vectors can be used to transfect COS cells by the DEAE-dextran method (Aruffo *et al.*, *Cell,* 61:1303-1313 (1990); Zettmeissl *et al.*, *DNA Cell Biol.*, (US) 9:347-353 (1990)). If larger amounts of protein are desired, the immunoadhesin can be expressed after stable transfection of a host cell line. For example, a pRK5-based vector can be introduced into Chinese hamster ovary (CHO) cells in the presence of an additional plasmid encoding dihydrofolate reductase (DHFR) and conferring resistance to G418. Clones resistant to G418 can be selected in culture; these clones are grown in the presence of increasing levels of DHFR inhibitor methotrexate; clones are selected, in which the number of gene copies encoding the DHFR and immunoadhesin sequences is co-amplified. If the immunoadhesin contains a hydrophobic leader sequence at its N-terminus, it is likely to be processed and secreted by the transfected cells. The expression of immunoadhesins with more complex structures may require uniquely suited host cells; for example, components such as light chain or J chain may be provided by certain myeloma or hybridoma cell hosts (Gascoigne *et al.*, *Proc. Natl. Acad. Sci. USA*, 84:2936-2940 (1987); Martin *et al.*, *J. Virol.*, 67:3561-3568 (1993)).

Immunoadhesins can be conveniently purified by affinity chromatography. The suitability of protein A as an affinity ligand depends on the species and isotype of the immunoglobulin Fc domain that is used in the chimera. Protein A can be used to purify immunoadhesins that are based on human γ1, γ2, or γ4 heavy chains (Lindmark *et al.*, *J. Immunol. Meth.*, 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss *et al.*, *EMBO J.*, 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. The conditions for binding an immunoadhesin to the protein A or G affinity column are dictated entirely by the characteristics of the Fc domain; that is, its species and isotype. Generally, when the proper ligand is chosen, efficient binding occurs directly from unconditioned culture fluid. One distinguishing feature of immunoadhesins is that, for human γ1 molecules, the binding capacity for protein A is somewhat diminished relative to an antibody of the same Fc type. Bound immunoadhesin can be efficiently eluted either at acidic pH

polypeptide DNA sequence and expressing the resultant DNA fusion construct in appropriate host cells. Ordinarily, nucleic acid encoding the AL-2 (or a fragment thereof) will be fused at its 3' end to nucleic acid encoding the N-terminus of the tag polypeptide, however 5' fusions are also possible.

Epitope tagged AL-2 can be conveniently purified by affinity chromatography using the anti-tag antibody. The matrix to which the affinity antibody is attached is most often agarose, but other matrices are available (*e.g.* controlled pore glass or poly(styrenedivinyl)benzene). The epitope tagged AL-2 can be eluted from the affinity column by varying the buffer pH or ionic strength or adding chaotropic agents, for example.

In another embodiment of the invention, multimeric soluble ligands are prepared by expression as chimeric molecules utilizing flexible linker loops. A DNA construct encoding the chimeric protein is designed such that it expresses two or more soluble or extracellular domains fused together in tandem (*e.g.*, "head-to-head") by a flexible loop. This loop may be entirely artificial (*e.g.* polyglycine repeats interrupted by serine or threonine at a certain interval) or "borrowed" from naturally occurring proteins (*e.g.* the hinge region of hIgG). Molecules can be engineered in which the length and composition of the loop is varied, to allow for selection of molecules with desired characteristics. Although not wishing to be limited by theory, it is believed that membrane attachment of the ligands can facilitate ligand clustering, which in turn can promote receptor multimerization and activation. Thus, one means of obtaining biological activity of the soluble AL-2 is mimicking, in solution, membrane associated ligand clustering. Thus, a biologically active, clustered soluble Eph-family ligand comprises (soluble AL-2)$_n$, wherein the soluble AL-2 is the receptor-binding AL-2 extracellular domain and n is 2 or greater. For example, despite the fact that receptor phosphorylation is markedly induced by stimulating receptor expressing reporter cells with mammalian cells overexpressing membrane-linked forms of the ligands AL-1 or B61, there is little or no observable phosphorylation using soluble forms of these ligands. However, when secreted forms of B61 are myc-tagged and antibodies are used to cluster the ligands, or when AL-1-IgG chimera is used, the previously inactive soluble ligands strongly induce receptor tyrosine phosphorylation in reporter cells expressing Ehk-1 or Rek7 receptors, respectively. Dimerization of the soluble ligand, *e.g.*, utilizing Fc, can be sufficient for achieving a biological response, however, further clustering of the ligand according to the invention, for example using anti-Fc antibodies, may achieve an increase in biological activity. Cells of the present invention may transiently or, preferably, constitutively and permanently express AL-2 in native form, or in soluble form as chimeric tagged AL-2, AL-2 immunoadhesin, or clustered AL-2 as described herein.

Accordingly, a method of enhancing the biological activity of the soluble AL-2 or its ECD is provided that includes the steps of (a) expressing the soluble domain of AL-2 with an epitope tag and (b) exposing the tagged soluble domain to anti-tag antibodies. The position of the tag with respect to AL-2 is not important so long as the tag does not interfere with AL-2 function and, in turn, AL-2 does not interfere with tag function. The tag is preferably located at either termini of AL-2, more preferably at the C-terminus of AL-2. However, the tag may be attached by covalent means, including with oxime linkages as taught for example in WO 9425071 published November 11, 1994.

In additional embodiments are compounds of the formula (AL-2)$_n$X, where n is an integer greater than or equal to 2 and X is an organic linker covalently binding each AL-2. For example, AL-2 dimers and multimers can be made by attaching AL-2 peptides to an organic linker or baseplate (designated as X in the formula) using

To produce AL-2, an expression vector will contain nucleic acid that encodes AL-2 as described above. The AL-2 of this invention is expressed directly in recombinant cell culture, or as a fusion with a heterologous polypeptide, preferably a signal sequence or other polypeptide having a specific cleavage site at the junction between the heterologous polypeptide and the AL-2.

In one example of recombinant host cell expression, mammalian cells are transfected with an expression vector comprising AL-2 DNA and the AL-2 encoded thereby is recovered from the culture, preferably cell culture medium in which the recombinant host cells are grown. But the expression vectors and methods disclosed herein are suitable for use over a wide range of prokaryotic and eukaryotic organisms.

Prokaryotes may be used for the initial cloning of DNAs and the construction of the vectors useful in the invention. However, prokaryotes may also be used for expression of DNA encoding AL-2. Polypeptides that are produced in prokaryotic host cells typically will be non-glycosylated.

Plasmid or viral vectors containing replication origins and other control sequences that are derived from species compatible with the host cell are used in connection with prokaryotic host cells, for cloning or expression of an isolated DNA. For example, *E. coli* typically is transformed using pBR322, a plasmid derived from an *E. coli* species (Bolivar, *et al.*, *Gene*, 2:95-113 (1987)). PBR322 contains genes for ampicillin and tetracycline resistance so that cells transformed by the plasmid can easily be identified or selected. For it to serve as an expression vector, the pBR322 plasmid, or other plasmid or viral vector, must also contain, or be modified to contain, a promoter that functions in the host cell to provide messenger RNA (mRNA) transcripts of a DNA inserted downstream of the promoter (Rangagwala, *et al.*, *Bio/Technology*, 9:477-479 (1991)).

In addition to prokaryotes, eukaryotic microbes, such as yeast, may also be used as hosts for the cloning or expression of DNAs useful in the invention. Yeast, for example, *Saccharomyces cerevisiae*, is a commonly used eukaryotic microorganism. Plasmids useful for cloning or expression in yeast cells of a desired DNA are well known, as are various promoters that function in yeast cells to produce mRNA transcripts.

Furthermore, cells derived from multicellular organisms also may be used as hosts for the cloning or expression of DNAs useful in the invention. Mammalian cells are most commonly used, and the procedures for maintaining or propagating such cells *in vitro*, which procedures are commonly referred to as tissue culture, are well known. Kruse and Patterson, eds., *Tissue Culture* (Academic Press, 1977). Examples of useful mammalian cells are human cell lines such as 293, HeLa, and WI-38, monkey cell lines such as COS-7 and VERO, and hamster cell lines such as BHK-21 and CHO, all of which are publicly available from the American Type Culture Collection (ATCC), Rockville, Maryland 20852 USA.

Expression vectors, unlike cloning vectors, should contain a promoter that is recognized by the host organism and is operably linked to the AL-2 nucleic acid. Promoters are untranslated sequences that are located upstream from the start codon of a gene and that control transcription of the gene (that is, the synthesis of mRNA). Promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate high level transcription of the DNA under their control in response to some change in culture conditions, for example, the presence or absence of a nutrient or a change in temperature.

A large number of promoters are known, that may be operably linked to AL-2 DNA to achieve expression of AL-2 in a host cell. This is not to say that the promoter associated with naturally occurring AL-2

polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide: a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence: or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers are used, in conjunction with standard recombinant DNA methods.

Expression and cloning vectors also will contain a sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosome(s), and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (for example, from SV40, polyoma, or adenovirus) are useful for cloning vectors in mammalian cells. Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector may be cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

The expression vector may also include an amplifiable gene, such as that comprising the coding sequence for dihydrofolate reductase (DHFR). Cells containing an expression vector that includes a DHFR gene may be cultured in the presence of methotrexate, a competitive antagonist of DHFR. This leads to the synthesis of multiple copies of the DHFR gene and, concomitantly, multiple copies of other DNA sequences comprising the expression vector (Ringold *et al.*, *J. Mol. Apl. Genet.*, 1:165-175 (1981)), such as a DNA sequence encoding AL-2. In that manner, the level of AL-2 produced by the cells may be increased.

DHFR protein encoded by the expression vector also may be used as a selectable marker of successful transfection. For example, if the host cell prior to transformation is lacking in DHFR activity, successful transformation by an expression vector comprising DNA sequences encoding AL-2 and DHFR protein can be determined by cell growth in medium containing methotrexate. Also, mammalian cells transformed by an expression vector comprising DNA sequences encoding AL-2, DHFR protein, and aminoglycoside 3' phosphotransferase (APH) can be determined by cell growth in medium containing an aminoglycoside antibiotic such as kanamycin or neomycin. Because eukaryotic cells do not normally express an endogenous APH activity, genes encoding APH protein, commonly referred to as $neo^r$ genes, may be used as dominant selectable markers in a wide range of eukaryotic host cells, by which cells transfected by the vector can easily be identified or selected (Jiminez *et al.*, *Nature*, 287:869-871 (1980); Colbere-Garapin *et al.*, *J. Mol. Biol.*, 150:1-14 (1981); Okayama *et al.*, *Mol. Cell. Biol.*, 3:280-289 (1983)).

Many other selectable markers are known that may be used for identifying and isolating recombinant host cells that express AL-2. For example, a suitable selection marker for use in yeast is the trp1 gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, *Nature*, 282:39-43 (1979); Kingsman *et al.*, *Gene*, 7:141-152 (1979); Tschemper, *et al.*, *Gene*, 10:157-166 (1980)). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 (available

antibody by any particular method. Monoclonal antibodies include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an anti-AL-2 antibody with a constant domain (*e.g.* "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (*e.g.*, Fab, $F(ab')_2$, and Fv), so long as they exhibit the desired biological activity. (See, *e.g.*, Mage *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp.79-97 (Marcel Dekker, Inc., New York (1987)). The monoclonal antibodies to be used in accordance with the present invention can be made by hybridoma method known in the art, or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage libraries generated using the techniques described in McCafferty *et al.*, *Nature*, 348:552-554 (1990), for example. The individual antibodies comprising the monoclonal antibody population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Humanized" forms of non-human (*e.g.* murine) antibodies are specific chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

DNA encoding the monoclonal antibodies of the invention is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA.

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (US Patent 4,816,567 by Cabilly *et al.*) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human

(providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published March 3, 1994. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

Competitive binding assays rely on the ability of a labeled standard (*e.g.*, AL-2 or an immunologically reactive portion thereof) to compete with the test sample analyte (AL-2) for binding with a limited amount of antibody. The amount of AL-2 in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three part complex (for example, see U.S. 4,376,110). The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

AL-2 antibodies may be useful in certain therapeutic indications to block activity of the AL-2 (for example in carcinogenesis).

Therapeutic AL-2 antibody formulations and modes for administration will be similar to those described herein for AL-2. A typical daily dosage of the antibody ranges from about 1 μg/kg to up to 5 mg/kg or more, depending on the factors mentioned herein for AL-2 administration.

AL-2 antibodies may also be useful in diagnostic assays for AL-2, *e.g.*, detecting its expression in specific cells, tissues, or serum. The antibodies are labeled in the same fashion as AL-2 described above and/or are immobilized on an insoluble matrix. AL-2 antibodies also are useful for the affinity purification of AL-2 from recombinant cell culture or natural sources. AL-2 antibodies that do not detectably cross-react with other proteins can be used to purify AL-2 free from these other known proteins. Suitable diagnostic assays for AL-2 and its antibodies are described herein.

The anti-AL-2 antibodies of the invention also are useful for *in vivo* imaging, wherein an antibody labeled with a detectable moiety is administered to a host, preferably into the bloodstream, and the presence and location of the labeled antibody in the host is assayed. This imaging technique is useful in the staging and

not activate the receptor. In this instance, the ligand may act as an antagonist for other molecules which activate the receptor and induce signal transduction.

The invention provides a method of modulating the endogenous enzymatic activity of an AL-2-binding Eph-family receptor. The method includes the step of administering to a mammal an effective amount of AL-2 to modulate the receptor enzymatic activity. In one embodiment is provided a method for stimulating the proliferation, differentiation, metabolism, regeneration, growth, process-out growth, or cell migration of AL-2-receptor expressing cells in a mammal by administering a therapeutically effective amount of receptor-activating AL-2. Receptor-activating forms of AL-2, such as AL-2-IgG, find use in alleviating cell damage or promoting neurogenesis following disease or injury, such as cytotoxicity, caused by chemotherapy. For example, a method for stimulating proliferation of neurons innervating the liver includes the step of administering a therapeutically effective amount of AL-2. Treatment with AL-2 is useful for repairing liver damage resulting from disease or injury.

Soluble Eph-family-receptor polypeptides can be used to modulate the activation of the cell-associated receptors, typically by competing with the cell-bound receptor for unbound AL-2. Modulation of Eph-family receptor activation may in turn alter the proliferation and/or differentiation of receptor-bearing cells.

Antibodies to Eph-like receptors are useful reagents for the detection of receptors in different cell types using immunoassays conventional to the art. Antibodies are also useful therapeutic agents for modulating receptor activation. Antibodies may bind to the receptor so as to directly or indirectly block ligand binding and thereby act as an antagonist of receptor activation. Alternatively, antibodies may act as an agonist by binding to receptor so as to facilitate ligand binding and bring about receptor activation at lower ligand concentrations. In addition, antibodies can themselves act as a ligand by inducing receptor activation. In this context the present invention provides anti-idiotype antibodies, *i.e.*, anti-AL-2-antibodies, that recognize an AL-2-binding-Eph-family receptor.

Accordingly, a method for modulating the activation of an AL-2-binding-Eph-family receptor by administering a modulation-effective amount of AL-2 or soluble AL-2. The term "modulation-effective amount" is that amount which effects an increase or decrease in the activation of an AL-2-binding-Eph-family receptor. Preferably the amount will range from about 0.01 $\mu$g to about 100 mg of polypeptide per kg body weight. In general, for therapeutic purposes, therapy will be appropriate for a patient having a condition in part related to the state of proliferation and/or differentiation of receptor-bearing cells. Based in part upon the tissue distribution of AL-2, and thus presumably its receptors in some embodiments, treatment with the pharmaceutical compositions of the invention may be particularly indicated for disorders involving brain, heart, muscle, lung, kidney, pancreas, skeletal muscle, liver, and more preferably involving brain, pancreas, and skeletal muscle.

AL-2 is also useful for selection of cell populations enriched for AL-2-receptor bearing cells. Such populations can be useful in cellular therapy regimens where it is necessary to treat patients that are depleted of certain cell types.

The human AL-2 is clearly also useful insofar as it can be administered to a human having depressed levels of endogenous AL-2, preferably in the situation where such depressed levels lead to a pathological disorder.

receptors and cortical neurons that express AL-2. AL-2 can be used in bacterial and viral infections of the nervous system, deficiency diseases, such as Wernicke's disease and nutritional polyneuropathy, progressive supranuclear palsy, Shy Drager's syndrome, multistem degeneration and olivo ponto cerebellar atrophy, and peripheral nerve damage.

For example, in Alzheimer's disease there is a critical loss of basal forebrain cholinergic neurons, cortical neurons, and hippocampal neurons. Although maximally effective treatment of this neurodegenerative condition may require protection of all vulnerable neuronal populations, treatment with AL-2 alone is expected to provide therapeutic benefit. Alzheimer's disease, which has been identified by the National Institutes of Aging as accounting for more than 50% of dementia in the elderly, is also the fourth or fifth leading cause of death in Americans over 65 years of age. Four million Americans, 40% of Americans over age 85 (the fastest growing segment of the U.S. population), have Alzheimer's disease. Twenty-five percent of all patients with Parkinson's disease also suffer from Alzheimer's disease-like dementia. And in about 15% of patients with dementia, Alzheimer's disease and multi-infarct dementia coexist. The third most common cause of dementia, after Alzheimer's disease and vascular dementia, is cognitive impairment due to organic brain disease related directly to alcoholism, which occurs in about 10% of alcoholics. However, the most consistent abnormality for Alzheimer's disease, as well as for vascular dementia and cognitive impairment due to organic brain disease related to alcoholism, is the degeneration of the cholinergic system arising from the basal forebrain (BF) to both the codex and hippocampus (Bigl *et al.*, in *Brain Cholinergic Systems*, M. Steriade and D. Biesold, eds., Oxford University Press, Oxford, pp.364-386 (1990)). And there are a number of other neurotransmitter systems affected by Alzheimer's disease (Davies, *Med. Res. Rev.*, 3:221 (1983)). However, cognitive impairment, related for example to degeneration of the cholinergic neurotransmitter system, is not limited to individuals suffering from dementia. It has also been seen in otherwise healthy aged adults and rats. Studies that compare the degree of learning impairment with the degree of reduced cortical cerebral blood flow in aged rats show a good correlation (Berman *et al.*, *Neurobiol. Aging*, 9:691 (1988)). In chronic alcoholism the resultant organic brain disease, like Alzheimer's disease and normal aging, is also characterized by diffuse reductions in cortical cerebral blood flow in those brain regions where cholinergic neurons arise (basal forebrain) and to which they project (cerebral cortex) (Lofti *et al.*, *Cerebrovasc.and Brain Metab. Rev.* 1:2 (1989)).

The progressive nature of Parkinson's disease is due to a loss of nigral dopaminergic neurons of the substantia nigra (Studer *et al.*, *Eur. J. Neuroscience*, 7:223-233 (1995)). ALS involves progressive degeneration of motoneurons of the spinal cord, brain stem and cerebral cortex.

Further, AL-2 can be used to treat neuropathy, and especially peripheral neuropathy. "Peripheral neuropathy" refers to a disorder affecting the peripheral nervous system, most often manifested as one or a combination of motor, sensory, sensorimotor, or autonomic neural dysfunction. The wide variety of morphologies exhibited by peripheral neuropathies can each be attributed uniquely to an equally wide number of causes. For example, peripheral neuropathies can be genetically acquired, can result from a systemic disease, or can be induced by a toxic agent. Examples include but are not limited to distal sensorimotor neuropathy, or autonomic neuropathies such as reduced motility of the gastrointestinal tract or atony of the urinary bladder. Examples of neuropathies associated with systemic disease include post-polio syndrome; examples of hereditary neuropathies include Charcot-Marie-Tooth disease, Refsum's disease, Abetalipoproteinemia, Tangier disease,

factors which induce endothelial cell migration, proliferation, and capillary formation. Numerous factors have been identified which induce vessel formation *in vitro* or *in vivo* in animal models. These include FGFα, FGFβ, TGF-α, TNF-α, VPF or VEGF, monobutyrin, angiotropin, angiogenin, hyaluronic acid degradation products, and more recently, B61 for TNF-α-induced angiogenesis (Pandey *et al.*, *Science.* 268:567-569 (1995)). Inhibitors of angiogenesis include a cartilage-derived inhibitor identified as TIMP, PF-4, thrombospondin, laminin peptides, heparin/cortisone, minocycline, fumagillin, difluoromethyl ornithine, sulfated chitin derivatives, and B61 antibody. The major development of the vascular supply occurs during embryonic development, at ovulation during formation of the corpus luteum, and during wound and fracture healing. Many pathological disease states are characterized by augmented angiogenesis including tumor growth, diabetic retinopathy, neovascular glaucoma, psoriasis, and rheumatoid arthritis. During these processes normally quiescent endothelial cells which line the blood vessels sprout from sites along the vessel, degrade extracellular matrix barriers, proliferate, and migrate to form new vessels. Angiogenic factors, secreted from surrounding tissue, direct the endothelial cells to degrade stromal collagens, undergo directed migration (chemotaxis), proliferate, and reorganize into capillaries.

AL-2 may stimulate either the growth or differentiation of cells expressing an AL-2 receptor. AL-2 that induces differentiation of AL-2-receptor bearing may be useful in the treatment of certain types of cancers. AL-2 may be used alone or in combination with standard chemotherapy or radiation therapy for cancer treatment. Where an AL-2-receptor is shown to be involved in the development of a cancerous state, either through stimulation of cell growth or through promotion of metastasis by stimulating cell mobility and adhesion, AL-2 antagonists as taught herein will find use. Fragments or analogs of AL-2 that bind to but do not activate the receptor are useful antagonists. Administration of an antagonist having affinity for the receptor will block receptor binding and activation by endogenous activators. Administration of soluble AL-2 receptor may also be used to counteract the biological effects of receptor activation. Soluble AL-2 receptor will compete with endogenous cell surface receptors for binding to activators, including AL-2, and thereby reduce the extent of AL-2 receptor activation. In addition, monoclonal antibodies directed either to AL-2 or to the receptor may be useful in blocking the interactions of AL-2, or other activator, with AL-2 receptors on cell surfaces.

Accordingly, AL-2 can find further use in promoting or enhancing angiogenesis by receptor activation on endothelial or stromal cells. The induction of vascularization is a critical component of the wound healing process. Neovascularization, also known as angiogenesis, is a complex process involving several sequential steps including basement membrane degradation, endothelial cell mobilization and proliferation, vessel canalization, and new basement membrane formation (Mantovani, *Int. J. Cancer,* 25:617 (1980)). Vascularization ensures that proliferating and differentiating fibroblasts are supplied with nutrients and oxygen, and that elements of humoral and cellular immunity are delivered to sites of potential bacterial infection. It is desirable to induce neovascularization as early as possible in the course of wound healing, particularly in the case of patients having conditions that tend to retard wound healing, *e.g.*, burns, decubitus ulcers, diabetes, obesity and malignancies. Even normal post-surgical patients will be benefited if they can be released from hospital care at any earlier date because of accelerated wound healing. This invention provides novel compositions and methods for modulating angiogenesis. A patient bearing a wound can be treated by applying an angiogenically active dose of an AL-2 compound to the wound. This facilitates the neovascularization of surgical incisions,

wound surface and the adsorbent substance in the dressing, the membrane containing pores sufficiently small for AL-2 to diffuse into the wound but not sufficiently large for epithelial cells to penetrate into the adsorbent. The degree of adsorbency will vary considerably and in fact dressings are included herein which are nonadsorbent, *i.e.*, the AL-2 is deposited or stored in an aqueous reservoir which is used to irrigate the wound on a continuous or intermittent basis.

AL-2 also is formulated into ointments or suspensions, preferably in combination with purified collagen, in order to produce semisolid or suspension vehicles. Conventional oleaginous formulations containing AL-2 are useful as salves. Such AL-2 carriers and formulations release AL-2 on a sustained basis at the wound, thereby serving to create a chemotactic gradient that directionally orients neovascularization, *e.g.*, into a skin graft. Sustained release formulations for AL-2 include semipermeable polymer matrices in the form of shaped articles, *e.g.*, films, or microcapsules. Implantable sustained release matrices include copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, *Biopolymers*, 22(1):547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, *J. Biomed. Mater. Res.*, 15:167-277 (1981) and Langer, *Chem. Tech.*, 12:98-105 (1982)), ethylene vinyl acetate (Langer *et al.*, Id.), or poly-D-(-)-3-Hydroxybutyric acid (EP 133,988A). These formulations may function as bioerodible matrices or as stable sources for the passive diffusion of AL-2.

Sustained release AL-2 compositions for contact with wounds also include liposomally entrapped AL-2. Liposomes containing AL-2 are prepared by methods known per se: DE 3,218,121A; Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688-3692 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4030-4034 (1980); EP 52322A; EP 36676A; EP 88046A; EP 143949A; EP 142641A; Japanese patent application 83-118008; U.S. patents 4,485,045 and 4,544,545; and EP 102,324A. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal rate of AL-2 leakage.

AL-2 is formulated with other ingredients such as carriers and/or adjuvants, *e.g.*, albumin, nonionic surfactants and other emulsifiers. There are no limitations on the nature of such other ingredients, except that they must be pharmaceutically acceptable, efficacious for their intended administration, and cannot degrade the activity of the active ingredients of the compositions. Suitable adjuvants include collagen or hyaluronic acid preparations, fibronectin, factor XIII, or other proteins or substances designed to stabilize or otherwise enhance the active therapeutic ingredient(s).

AL-2 optionally is supplied with other known angiogenic agents such as heparin, which has been shown to accelerate the healing of thermal burns, TNF, TGF-$\alpha$, TGF-$\beta$, fibroblast growth factor, epidermal growth factor, B61, angiogenin, platelet factor 4, insulin, PDGF, and angiogenesis factor and the angiogenic activity of the combinations observed for synergistic effects. AL-2 optionally also is combined with an with an IFN, *e.g.*, IFN-$\gamma$, and other cytokines, or may be free of interferons such as IFN-$\gamma$. Where such cytokines or known angiogenic agents are species-specific, the appropriate cytokine or agent will be selected for the species to be treated.

Animals or humans are treated in accordance with this invention. It is possible but not preferred to treat an animal of one species with AL-2 of another species. A preferred AL-2 for use herein is soluble AL-2-IgG.

The amount of AL-2 to be contacted with the wound depends upon a great number of variables that will be taken into account by the clinician, including the presence of other angiogenic agents in the AL-2

AL-2 antagonists can find use in inhibiting, preventing or treating pathological angiogenesis, such as during tumor vascularization. Tumor neovascularization is a vital stage in the growth of solid tumors (Polverini *et al.*, *Lab. Invest.*, 51:635-642 (1985)). The progressive growth of solid tumors is strictly dependent on their ability to attract new blood vessels that will supply them with oxygen and essential nutrients (Bouck, *Cancer Cells.* 2(6):179-185 (1990)). Angiogenesis has been shown to precede or accompany malignancy. In the absence of neovascularization the size of tumor grafts becomes limited. When angiogenesis is absent, tumors tend to remain dormant. Therefore, angiogenic activity has been directly correlated with tumor growth. AL-2 antagonist compositions and methods of the invention can modulate (*e.g.*, prevent or reduce) new capillary growth into tumors.

A variety of non-neoplastic diseases, previously thought to be unrelated, can be considered "angiogenic diseases" because they are dominated by the pathological growth of capillary blood vessels. These diseases include diabetic retinopathy, arthritis, hemangiomas, psoriasis, and ocular neovascularization. AL-2 antagonist compositions and methods of the invention can be used to treat these conditions.

Vascularization also plays a critical role in chronic inflammatory conditions such as rheumatoid arthritis (Koch *et al.*, *Arthr. Rheum.*, 29:471-479 (1986)). Rheumatoid arthritis ("RA") is a chronic heterogeneous disorder in which a variety of etiological agents may be responsible for initiating a series of events leading to inflammation in multiple joints. The cause of the disease remains unknown, although by analogy with other forms of arthritis such as that accompanying Lyme disease, it has been postulated that infection with as yet unidentified bacteria or viruses in a genetically susceptible host is an initiating event. Persistence could result from the presence of viral or bacterial antigens that generate an immune response or cross-react with host tissues together with amplification effects of cellular products of the host. While many patients have systemic manifestations in RA, many of the most serious consequences of RA stem from its effects on articular connective tissues, which are characterized by alterations of the synovial membrane with proliferation of lining cells and infiltration by chronic inflammatory cells. Erosions of bone occur in areas contiguous with the inflammatory cell mass as well as in regions adjacent to bone marrow distant from the inflammation. The bone erosions are probably produced through induction of differentiation and activation of osteoclast progenitors. The erosion of soft connective tissues, *e.g.*, cartilage, joint capsules, tendons, and ligaments, results from direct release of proteolytic enzymes from cells of the inflammatory cell mass or from polymorphonuclear leukocytes that are typically abundant in rheumatoid synovial fluids, although rare in the synovial membrane. See, for example, Harris, W. N. Kelley *et al.*, eds., *Textbook of Rheumatology*, W.B. Saunders, Philadelphia, pp. 886-915 (1985); Dayer *et al.*, *Clin. Rheum. Dis.*, 4:517-537 (1978); Krane, *Arthritis and Allied Conditions. A textbook of Rheumatology*, ed. by D.J. McCarty, pp. 593-604, Lea and Febiger, Philadelphia (1985); and Krane *et al.*, *Lymphokines*, 7:75-136 (1982). Therapy for RA depends on the stage of the disease. Stage 1, where a postulated antigen is presented to T-cells with no obvious arthritic symptoms, is not treated. Stage 2 involves T-cell and B-cell proliferation and angiogenesis in synovial membrane, resulting in malaise, mild joint stiffness, and swelling. During Stage 3, neutrophils accumulate in synovial fluid and synovial cells proliferate without polarization or invasion of cartilage, resulting in joint pain and swelling, morning stiffness, malaise, and weakness. Current therapy for Stages 2 and 3 includes bed rest, application of heat, supplemental eicosapentaenoic and docosahexanoic acid, and drugs. Nonsteroidal anti-inflammatory drugs, including aspirin, continue to be the foundation of drug

retinopathy, tumors such as malignant tumors (*e.g.*, cancer such as mastocarcinoma, hepatoma, colic carcinoma, Kaposi's sarcoma, lung carcinomas and other epithelial carcinomas).

Numerous methods, *in vitro* and *in vivo*, are available to screen candidate AL-2 or AL-2 antagonists compounds for angiogenic or angiogenesis-inhibiting properties. Several *in vitro* assays of endothelial cell growth, migration, and capillary tube formation are known and can be used with the compounds of the invention, particularly as initial screening methods for angiogenic or angiostatic substances. Further testing would typically use *in vivo* animal testing. U.S. Patent 5,382,514, which is incorporated herein, describes numerous models for angiogenesis *in vivo*. For example, the corneal pocket assay involves the surgical implantation of polymer pellets containing angiogenic factors in the cornea of larger animals such as rabbits. Since quantitation can be difficult the assay is usually used for preferred candidate compounds. The rabbit ear chamber assay requires the surgical insertion of a glass or plastic viewing device and measurement of capillary migration by microscopy. The rat dorsal air sac assay involves implants of stainless steel chambers containing angiogenic factors. An alginate assay which generates an angiogenic response has been described which involves the injection of tumor cells encased in alginate subcutaneously into mice. The accumulation of hemoglobin in the injected gel is used to quantitate the angiogenic response. A compound can be administered to the chorio-allantoic membranes of aged, typically three-day-aged, fertilized chicken eggs and the appearance of neovascularization after a lapse of time, typically two days is observed (CAM assay; Ausprunk *et al.*, *Am. J. Pathol.*, 97:597 (1975)). The neovascularization inhibitory rates are compared with an untreated control group. A more recent assay method involves providing a liquid matrix material which forms a matrix gel when injected into a host; adding an angiogenic agent to the liquid matrix material; injecting the liquid matrix material containing the angiogenic agent into a host to form a matrix gel; recovering the matrix gel from the host; and quantitating angiogenesis of the recovered matrix gel. A variation of this can be used to test for inhibitors of vascularization in a tissue by providing a liquid matrix material which forms a matrix gel when injected into a host; adding an angiogenic inhibiting agent to the liquid matrix material; and injecting the liquid matrix material containing the angiogenic inhibiting agent into a tissue situs of a host to form a matrix gel. This system can be used with compounds of the invention when inducing vascularization in a tissue is desired by providing a liquid matrix material which forms a matrix gel when injected into a host; adding an angiogenic inducing agent to the liquid matrix material; and injecting the liquid matrix material containing the angiogenic inducing agent into a tissue situs of a host to form a matrix gel. In a preferred embodiment, a solution of basement membrane proteins supplemented with fibroblast growth factor and heparin is injected subcutaneously in a host, *e.g.*, a mouse, where it forms a gel. Sprouts from vessels in the adjacent tissue penetrate into the gel within days connecting it with the external vasculature. Angiogenesis is then quantitated by image analysis of vessels and by measuring the hemoglobin present in the vessels within the gel. This assay method facilitates the testing of both angiogenic and angiostatic agents *in vivo*. In addition, the endothelial cells responding to the angiogenic factors can be recovered *in vitro* for further studies. Preferred compounds have 50-70% inhibition rates, and more preferred compounds show 80-100% inhibition rates. As described herein the angiogenically active proteins of the invention provide use in *in vitro* and *in vivo* screens for compounds that inhibit angiogenesis by measuring inhibition of AL-2 stimulated angiogenesis in the presence and absence of the candidate inhibitor.

into the tissue of a patient, or may be encapsulated within porous membranes which are then implanted in a patient, in either case providing for the delivery of AL-2 or anti-AL-2 antagonist into areas within the body of the patient in need of increased or decreased concentrations of AL-2. Alternatively, an expression vector comprising AL-2 DNA may be used for *in vivo* transformation of a patient's cells to accomplish the same result.

An effective amount of AL-2 or AL-2 antagonist, *e.g.*, anti-AL-2 antibody, to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage might range from about 1 μg/kg to up to 100 mg/kg or more, depending on the factors mentioned above. Where possible, it is desirable to determine appropriate dosage ranges first *in vitro*, for example, using assays for neuronal cell survival or growth which are known in the art, and then in suitable animal models, from which dosage ranges for human patients may be extrapolated. In a specific embodiment of the invention, a pharmaceutical composition effective in promoting the survival or growth of neurons will provide a local growth promoting activity concentration *in vivo* of between about 0.1 and 10 ng/ml. Typically, the clinician will administer AL-2 until a dosage is reached that achieves the desired effect. Therapeutic progress is easily monitored by conventional assays.

In the treatment of tumors the compositions described herein can be administered subcutaneously or intramuscularly, for example, and the pharmacological activities of an AL-2 antagonist can be maintained over a long period of time by the sustained-release effect of a composition of the present invention. The number of administrations can therefore be reduced. The composition can also be by directly injecting the composition into a tumor-controlling artery. In the case of treatment of an adult patient having a tumor, the dose of the AL-2 antagonist can be appropriately selected depending upon the kind of tumor, site, size, and kind of AL-2 antagonist. For example, the dose of a protein AL-2 antagonists, particularly an antibody, can be about 0.1 mg to about 500 mg, typically about 1.0 mg to about 300 mg, more typically about 25 mg to about 100 mg. The administration frequency can be appropriately selected depending upon the kind of disease and dosage form. In the case of injection into the tumor-controlled artery or tumor itself, frequently repeated injections are not required and a single injection once every one to 4 weeks may be sufficient for the desired therapeutic effects.

The nucleic acid encoding the AL-2 may be used as a diagnostic for tissue-specific typing. For example, such procedures as *in situ* hybridization, Northern and Southern blotting, and PCR analysis, can be used to determine whether DNA and/or RNA encoding AL-2 is present in the cell type(s) being evaluated. AL-2 nucleic acid or polypeptide may also be used as diagnostic markers for such tissues. For example, the AL-2 may be labeled, using the techniques described herein, and expression of AL-2-receptor, including the preferred receptors disclosed herein, receptor can be quantified via its binding to labelled AL-2.

AL-2 nucleic acid is also useful for the preparation of AL-2 polypeptide by recombinant techniques exemplified herein.

The invention also provides methods for studying the function of the AL-2 protein. Cells, tissues, and non-human animals lacking AL-2 expression, partially lacking in AL-2 expression, or over-expressing AL-2 can be developed using recombinant molecules of the invention having specific deletion or insertion mutations in the AL-2 gene. For example, the extracellular domain or parts thereof, the transmembrane region or parts

The AL-2 proteins disclosed herein can be employed to measure the biological activity of an AL-2 receptor in terms of binding affinity for AL-2. For example, AL-2 can be employed in a binding affinity study to measure the biological activity of a receptor that has been stored at different temperatures, or produced in different cell types. Thus, AL-2 proteins find use as reagents in "quality assurance" studies, *e.g.*, to monitor shelf life and stability of receptor protein under different conditions. Furthermore, AL-2 can be used in determining whether biological activity is retained after modification of a receptor protein (*e.g.*, chemical modification, truncation, mutation, etc.). The binding affinity of the modified receptor for an AL-2 is compared to that of an unmodified receptor to detect any adverse impact of the modifications on biological activity of the receptor.

Binding of AL-2 to an Eph-family receptor can be determined using conventional techniques, including competitive binding methods, such as RIAs, ELISAs, and other competitive binding assays. Ligand/receptor complexes can be identified using such separation methods as filtration, centrifugation, flow cytometry (see, *e.g.*, Lyman *et al.*, *Cell* 75:1157-1167 (1993); Urdal *et al.*, *J. Biol. Chem.* 263:2870-2877 (1988); and Gearing *et al.*, *EMBO J.* 8:3667-3676 (1989)). Results from binding studies can be analyzed using any conventional graphical representation of the binding data, such as Scatchard analysis (Scatchard, *Ann. NY Acad. Sci.* 51:660-672 (1949); and Goodwin *et al.*, *Cell* 73:447-456 (1993)), and the like. Since the AL-2 induces receptor phosphorylation, conventional tyrosine phosphorylation assays can also be used.

Isolated AL-2 polypeptide may be used in quantitative diagnostic assays as a standard or control against which samples containing unknown quantities of AL-2 may be prepared.

AL-2 preparations are also useful in generating antibodies, as standards in assays for AL-2 (*e.g.*, by labeling AL-2 for use as a standard in a radioimmunoassay, or enzyme-linked immunoassay), for detecting the presence of an AL-2-receptor in a biological sample (*e.g.*, using a labelled AL-2), in affinity purification techniques, and in competitive-type receptor binding assays when labeled for example with radioiodine, enzymes, fluorophores, spin labels, or branched DNA.

AL-2 polypeptide can be produced in prokaryotic cells using the techniques taught herein, and the unglycosylated protein so produced can be used as a molecular weight marker, for example. Preferably unglycosylated, soluble AL-2 is used. AL-2 can be used as a molecular weight marker in gel filtration chromatography or SDS-PAGE, for example, either analytical or preparative modes, where it is desirable to determine molecular weight(s) for separated peptides. AL-2 is most preferably used in combination with other known molecular weight markers as standards to provide a range of molecular weights. Other known molecular weight markers can be purchased commercially, *e.g.*, from Amersham Corporation, Arlington Heights, IL, for example. The molecular weight markers can be labelled to enable easy detection following separation. Techniques for labelling antibodies and proteins are discussed herein and are well known in the art. For example, the molecular weight markers can be biotinylated and, following separation on SDS-PAGE, for example, can be detected using streptavidin-horseradish peroxidase.

AL-2 is used for competitive screening of potential agonists or antagonists for binding to an AL-2 receptor. AL-2 variants are useful as standards or controls in assays for AL-2, provided that they are recognized by the analytical system employed, *e.g.*, an anti-AL-2 antibody.

cis-diaminodichloroplatinum, antimetabolites such as 5-fluorouracil, vinca alkaloids such as vincristine, and antibiotics such as bleomycin, doxorubicin, daunorubicin, and derivatives thereof. Among the toxins are ricin, abrin, diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A, ribosomal inactivating proteins, mycotoxins such as trichothecenes, and derivatives and fragments (*e.g.*, single chains) thereof. Radionuclides suitable for diagnostic use include, but are not limited to, $^{123}I$, $^{131}I$, $^{99m}Tc$, $^{111}In$, and $^{76}Br$. Radionuclides suitable for therapeutic use include, but are not limited to, $^{131}I$, $^{211}At$, $^{77}Br$, $^{186}Re$, $^{188}Re$, $^{212}Pb$, $^{212}Bi$, $^{109}Pd$, $^{64}Cu$, and $^{67}Cu$.

Such agents may be attached to AL-2 by any suitable conventional procedure. AL-2 contains functional groups on amino acid side chains that can be reacted with functional groups on a desired agent to form covalent bonds, for example. Alternatively, the protein or agent may be derivatized to generate or attach a desired reactive functional group, preferably a site-specific reactive group. The derivatization may involve attachment of one of the bifunctional coupling reagents available for attaching various molecules to proteins (Pierce Chemical Company, Rockford, Illinois). A number of techniques for radiolabeling proteins are known. Radionuclide metals may be attached to AL-2 by using a suitable bifunctional chelating agent, for example.

Conjugates comprising AL-2 and a suitable diagnostic or therapeutic agent (preferably covalently linked) are administered or otherwise employed in an amount appropriate for the particular application.

In view of the sequence identity between AL-2 and Htk-L as shown in Figures 4 and 5, which is determined for the first time herein, and since Htk-L is a ligand of the transmembrane-sequence type and binds an Eph-family receptor, namely Htk, the present application provides embodiments of methods of treatment wherein an effective amount of Htk-L is administered to a patient in need of such treatments as discussed for the first time herein for AL-2. Accordingly, WO 96/02645, published February 1, 1996, is incorporated by reference herein for its teachings regarding nucleic acid sequences encoding Htk-L, Htk-L proteins and variants, and methods for their production and formulation. Consequently, it is the intent of the present inventors that new uses and methods of administration of AL-2, as taught for the first time herein, are to be applied to Htk-L. For example, in one embodiment Htk-L will find use in methods of treatment of angiogenesis-related conditions as taught herein for AL-2.

In summary, by providing nucleic acid molecules encoding AL-2, the present invention enables for the first time the production of AL-2 by recombinant DNA methods, thus providing a reliable source of sufficient quantities of AL-2 for use in various diagnostic and therapeutic applications. In view of its distinct biological properties, purified recombinant AL-2 will be especially useful in a variety of circumstances, such as in angiogenesis-related conditions and where it is necessary or desirable to assure neuronal function, growth, survival, or cell-cell contact, but where other neurotrophic factors or angiogenic agents either cannot be used or are less effective.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated.

## EXAMPLES

### Example 1

Isolation of a full-length cDNA encoding AL-2.

981 (1995)). Conditioned media is collected after 3 days and AL-2-IgG is purified by Protein A chromatography.

**Example 4**

Biological Activity: Activation of Eph-Related Receptors by AL-2

The ability of AL-2 or variant, e.g., AL-2-IgG, to activate a Eph-family receptor can be determined by tyrosine autophosphorylation of the receptor in a receptor-expressing cell source as described herein. Cells expressing an Eph-family receptor, preferably Hek2, Hek5, Hek6/elk/Cek6, or Htk, are incubated with AL-2 and specific phosphorylation of the Eph-family receptor is monitored. Specific phosphorylation indicates that AL-2 not only binds to the Eph-family receptor, but that it also activates the Eph-family receptor.

Cells expressing an Eph-family receptor, e.g., cultured primary cortical neurons, are detected and analyzed by *in situ* hybridization and/or immunoprecipitation and immunoblotting with anti-Eph-family-receptor antibodies, preferably anti-Hek2, anti-Elk/Hek6/Cek6, anti-Hek5 or anti-Htk, and anti-phosphotyrosine antibodies. Membrane-bound AL-2 is transiently expressed on the surface of transfected 293 cells and its activation of the endogenous Eph-family receptor in the receptor-expressing cell is monitored. Activation of endogenous Eph-family receptor is indicated by autophosphorylation of the receptor. Alternatively, soluble AL-2 fusion, e.g., AL-2-IgG, dimers, multimers, as taught herein, is provided to the receptor-expressing cells and tested for activation of endogenous Eph-family receptor as described, for example, by Winslow *et al.*, *Neuron*, 14:973-981 (1995). Membrane-attachment has been reported as required or preferred for maximal receptor activation with other members of this ligand family (Davis *et al.*, *Science*, 266:816-819 (1994)).

HEK 293 cells are transfected with an AL-2 cDNA expression plasmid using the calcium phosphate coprecipitation method (Simonsen *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:2495-2499 (1983)). Primary cortical neurons from E16 rats are plated at a density of $5 \times 10^6$ cells /15 cm dish and cultured for 4 days. These cells are then treated with purified soluble AL-2 or soluble AL-2-IgG (0.1-1 μg/ml) or a number of 293 cells expressing an equivalent number of membrane-bound AL-2 for 10 min at 37 °C. Immunoprecipitation of lysates with rabbit anti-Eph-family receptors and immunoblotting with mouse anti-phosphotyrosine is essentially as described (Kaplan *et al.*, *Science*, 252:554-559 (1991); Kaplan *et al.*, *Nature*, 350:158-160 (1991)). Immunoblotted bands are visualized using a horseradish peroxidase-conjugated sheep anti-mouse antibody and the ECL fluorescence detection system (Amersham) as described by the manufacturer.

An ability of AL-2-IgG to activate the autophosphorylation of receptor, preferably to a similar extent as membrane-bound AL-2, indicates that the soluble AL-2-IgG fusion protein and the like can be used as an agonist for Eph-family receptors *in vitro*, *ex vivo*, and *in vivo*. An inability of soluble AL-2 (e.g., free ECD) to activate receptor autophosphorylation despite its ability to bind receptor indicates that certain soluble forms of AL-2 as taught herein can act as antagonists of Eph-family receptors.

(ix) FEATURE:
(A) NAME/KEY: signal peptide
(B) LOCATION: 244-321
(C) IDENTIFICATION METHOD:
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GNTCTAGAAN TAGTGGATCC CCCCGGGCTG CAGGAATTCC GACGGCCCCT 50

GGAAGGGCTC TGGTGGGGCT GAGCGCTCTG CCGCGGGGGC GCGGGCACAG 100

CAGGAAGCAG GTCCGCGTGG GCGCTGGGGG CATCAGCTAC CGGGGTGGTC 150

CGGGCTGAAG AGCCAGGCAG CCAAGGCAGC CACCCCGGGG GGTGGGCGAC 200

TTTGGGGGAG TTGGTGCCCC GCCCCCCAGG CCTTGGCGGG GTC ATG 246
Met
1

GGG CCC CCC CAT TCT GGG CCG GGG GGC GTG CGA GTC GGG 285
Gly Pro Pro His Ser Gly Pro Gly Gly Val Arg Val Gly
5 10

GCC CTG CTG CTG CTG GGG GTT TTG GGG CTG GTG TCT GGG 324
Ala Leu Leu Leu Leu Gly Val Leu Gly Leu Val Ser Gly
15 20 25

CTC AGC CTG GAG CCT GTC TAC TGG AAC TCG GCG AAT AAG 363
Leu Ser Leu Glu Pro Val Tyr Trp Asn Ser Ala Asn Lys
30 35 40

AGG TTC CAG GCA GAG GGT GGT TAT GTG CTG TAC CCT CAG 402
Arg Phe Gln Ala Glu Gly Gly Tyr Val Leu Tyr Pro Gln
45 50

ATC GGG GAC CGG CTA GAC CTG CTC TGC CCC CGG GCC CGG 441
Ile Gly Asp Arg Leu Asp Leu Leu Cys Pro Arg Ala Arg
55 60 65

CCT CCT GGC CCT CAC TCC TCT CCT AAT TAT GAG TTC TAC 480
Pro Pro Gly Pro His Ser Ser Pro Asn Tyr Glu Phe Tyr
70 75

AAG CTG TAC CTG GTA GGG GGT GCT CAG GGC CGG CGC TGT 519
Lys Leu Tyr Leu Val Gly Gly Ala Gln Gly Arg Arg Cys
80 85 90

GAG GCA CCC CCT GCC CCA AAC CTC CTT CTC ACT TGT GAT 558
Glu Ala Pro Pro Ala Pro Asn Leu Leu Leu Thr Cys Asp
95 100 105

CGC CCA GAC CTG GAT CTC CGC TTC ACC ATC AAG TTC CAG 597
Arg Pro Asp Leu Asp Leu Arg Phe Thr Ile Lys Phe Gln
110 115

GAG TAT AGC CCT AAT CTC TGG GGC CAC GAG TTC CGC TCG 636
Glu Tyr Ser Pro Asn Leu Trp Gly His Glu Phe Arg Ser

```
GTG AGT GGT GAC TAT GGG CAT CCT GTG TAT ATC GTG CAG 1221
Val Ser Gly Asp Tyr Gly His Pro Val Tyr Ile Val Gln
    315                 320                 325

GAT GGG CCC CCC CAG AGC CCT CCA AAC ATC TAC TAC ACA 1260
Asp Gly Pro Pro Gln Ser Pro Pro Asn Ile Tyr Tyr Thr
            330                 335

TCG ATT TCT GTG TTG GAG TGG CCC ATA TTG CAT ACG ATA 1299
Ser Ile Ser Val Leu Glu Trp Pro Ile Leu His Thr Ile
340                 345                 350

CAA CTG TTT TTC ATG CGA TCC AAG TGC TCC CGT GTC ACT 1338
Gln Leu Phe Phe Met Arg Ser Lys Cys Ser Arg Val Thr
        355                 360                 365

ACA TTC TTA TTT CCT GTG CAA GTT ATT ACG ACA TCG ACT 1377
Thr Phe Leu Phe Pro Val Gln Val Ile Thr Thr Ser Thr
                370                 375

TGC CGG ATG ACT TCA TTT AGC TTT ACC ACC CTG AAC CCA 1416
Cys Arg Met Thr Ser Phe Ser Phe Thr Thr Leu Asn Pro
    380                 385                 390

TCC ATG CAG GCC TGC AGA GCA CAG ATG GGG GAA TTC CGA 1455
Ser Met Gln Ala Cys Arg Ala Gln Met Gly Glu Phe Arg
            395                 400

ATC AGA TGG TGT TTC TGG GGG GAC AGG ATC CTG GGT ACG 1494
Ile Arg Trp Cys Phe Trp Gly Asp Arg Ile Leu Gly Thr
405                 410                 415

GCT CTG TTT GTG CTT GTG CTT ATT CTT CTT CTT GGG AGG 1533
Ala Leu Phe Val Leu Val Leu Ile Leu Leu Leu Gly Arg
        420                 425                 430

CTG AAT ATG CAT CAG ACG ACA CTG CTC CGG CAA CGG GCC 1572
Leu Asn Met His Gln Thr Thr Leu Leu Arg Gln Arg Ala
                435                 440

AGT GTG GAG GCG GAA GCC GGC CAG CAT GGT CCC CTG TG 1610
Ser Val Glu Ala Glu Ala Gly Gln His Gly Pro Leu
    445                 450                 455

ATAGGATTGA AAGAGCTACT GAGAATAGGG GGCTTCTCAA TGAGAGAGCG 1660

GAGGCTGCTG TTATCATGGG AACCAGGCAG ATCAATCATC CCTGGCAGGT 1710

CAGGCAGGAA GTTACTTAGC TTCTCCTTCA CCTTCTTCCC ACAGAATTTA 1760

TTATAGGCTT GTTCCAAGTT GTAGTGTGTG ATCAGATTCG TGCTGCCTGT 1810

CAGCTCTGTG CTACCTGGCA GTTCCCCTCA TGGAATTCGA TATCAAGCTT 1860

ATCGATACCG TCGACCT 1877
```

(2) INFORMATION FOR SEQ ID NO:2:

Ala Lys Pro Ser Glu Ser Arg His Pro Gly Pro Gly Ser Phe Gly
260 265 270

Arg Gly Gly Ser Leu Gly Leu Gly Gly Gly Gly Gly Met Gly Pro
275 280 285

Arg Glu Ala Glu Pro Gly Glu Leu Gly Ile Ala Leu Arg Gly Gly
290 295 300

Gly Ala Ala Asp Pro Pro Phe Cys Pro His Tyr Glu Lys Val Ser
305 310 315

Gly Asp Tyr Gly His Pro Val Tyr Ile Val Gln Asp Gly Pro Pro
320 325 330

Gln Ser Pro Pro Asn Ile Tyr Tyr Thr Ser Ile Ser Val Leu Glu
335 340 345

Trp Pro Ile Leu His Thr Ile Gln Leu Phe Phe Met Arg Ser Lys
350 355 360

Cys Ser Arg Val Thr Thr Phe Leu Phe Pro Val Gln Val Ile Thr
365 370 375

Thr Ser Thr Cys Arg Met Thr Ser Phe Ser Phe Thr Thr Leu Asn
380 385 390

Pro Ser Met Gln Ala Cys Arg Ala Gln Met Gly Glu Phe Arg Ile
395 400 405

Arg Trp Cys Phe Trp Gly Asp Arg Ile Leu Gly Thr Ala Leu Phe
410 415 420

Val Leu Val Leu Ile Leu Leu Leu Gly Arg Leu Asn Met His Gln
425 430 435

Thr Thr Leu Leu Arg Gln Arg Ala Ser Val Glu Ala Glu Ala Gly
440 445 450

Gln His Gly Pro Leu
455

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2380 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GNTCTAGAAN TAGTGGATCC CCCCGGGCTG CAGGAATTCC GACGGCCCCT 50

GGAAGGGCTC TGGTGGGGCT GAGCGCTCTG CCGCGGGGGC GCGGGCACAG 100

CAGGAAGCAG GTCCGCGTGG GCGCTGGGGG CATCAGCTAC CGGGGTGGTC 150

GCCCACCTTT TGGTTGGCAC CGCCTTCTTT CTGCCTCTCA CTGGTTTTCT 1650

CTTCTCTATC TCTTATTCTT TCCCTCTCTT CCGTCTCTAG GTCTGTTCTT 1700

CTTCCCTAGC ATCCTCCTCC CCACATCTCC TTTCACCCTC TTGGCTTCTT 1750

ATCCTGTGCC TCTCCCATCT CCTGGGTGGG GGCATCAAAG CATTTCTCCC 1800

CTTAGCTTTC AGCCCCCCTT CTGACCTCTC ATACCAACCA CTCCCCTCAG 1850

TCTGCCAAAA ATGGGGGCCT TATGGGGAAG GCTCTGACAC TCCACCCCAG 1900

CTCAGGCCAT GGGCAGCAGG GCTCCATTCT CTGGCCTGGC CCAGGCCTCT 1950

ACATACTTAC TCCAGCCATT TGGGGTGGTT GGGTCATGAC AGCTACCATG 2000

AGAAGAAGTG TCCCGTTTTG TCCAGTGGCC AATAGCAAGA TATGAACCGG 2050

TCGGGACATG TATGGACTTG GTCTGATGCT GAATGGGCCA CTTGGGACCG 2100

GAAGTGACTT GCTCCAGACA AGAGGTGACC AGGCCCGGAC AGAAATGGCC 2150

TGGGAAGTAG CAGAAGCAGT GCAGCAGGAA CTGGAAGTGC CTTCATCCAG 2200

GACAGGAAGT AGCACTTCTG AAACAGGAAG TGGTCTGGCT GGAACTCCAA 2250

GTGGCTTAGT CTGGGGGATC AGGAGGTGGG AGGTGGATGG TTCTTATTCT 2300

GTGGAGAAGA AGGGCGGGAA GAACTTCCTT TCAGGAGGAA GCTGGAACTT 2350

ACTGACTGTA AGAGGTTAGA GGTGGACCGA 2380

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 340 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gly Pro Pro His Ser Gly Pro Gly Gly Val Arg Val Gly Ala
1 5 10 15

Leu Leu Leu Leu Gly Val Leu Gly Leu Val Ser Gly Leu Ser Leu
20 25 30

Glu Pro Val Tyr Trp Asn Ser Ala Asn Lys Arg Phe Gln Ala Glu
35 40 45

Gly Gly Tyr Val Leu Tyr Pro Gln Ile Gly Asp Arg Leu Asp Leu
50 55 60

Leu Cys Pro Arg Ala Arg Pro Pro Gly Pro His Ser Ser Pro Asn
65 70 75

Tyr Glu Phe Tyr Lys Leu Tyr Leu Val Gly Gly Ala Gln Gly Arg
80 85 90

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCCGACGCTG TGAGGCACCC CCTGCCCCAA ACCTCCTTCT CACTTGTGAT 50

CGCCCAGACC TGGATCTCCG CTTCACCATC AAGTTCCAGG AGTATAGCCC 100

TAATCTCTGG GGCCACGAGT TCCGCTCGCA CCACGATTAC TACATCATTG 150

CCACATCGGA TGGGACCCGG GAGGCCTGGG AGAGCCTGCA GGGAAGTGTG 200

TGCCTAACCA GAGGCATGAA GGTGCTTCTC CGAGTNGGAC AAAGTCCCGA 250

GGAGGGGCTG TCCCCCGAAA ACCTGTGTCT GAAATGCCCA TGGAAAGAGA 300

CCGAGGGGCA GCCCACAGCC TGGGAGCCTG GGGAAGGAGA ACCTGCCAGG 350

TGACCCCACC AGCAATNCAA CCTTCCGGGG TTGCTTGAAG GGCCCCTTGA 400

CCCTTTCCCA GCATTGCNTG CANTTGGTTN GGGGCAGCAN GGGGGNGTTT 450

TGGC 454

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGACAAAGTC CCGAGGAGGG GCTGTCCCCC GAAAACCTGT GTCTGAAATG 50

CCCATGGAAA 60

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

**What is claimed is:**

1. An isolated nucleic acid, which encodes a polypeptide having an amino acid sequence that is at least 75% identical to the amino acid sequence for mature human AL-2 shown in Figure 1A-1B or Figure 2A-2B.

2. The isolated nucleic acid according to claim 1, which encodes a polypeptide having an amino acid sequence that is at least 85% identical to the amino acid sequence for mature human AL-2 shown in Figure 1A-1B or Figure 2A-2B.

3. The isolated nucleic acid of claim 2, comprising a nucleotide sequence encoding the amino acid sequence shown in Figure 1A-1B for mature AL-2l.

4. The isolated nucleic acid of claim 2, comprising a nucleotide sequence encoding the amino acid sequence shown in Figure 2A-2B for mature AL-2s.

5. The isolated nucleic acid of claim 2, which encodes a polypeptide having an amino acid sequence that is at least 75% homologous to the amino acid sequence of the extracellular domain of AL-2 shown in Figure 1A-1B.

6. The isolated nucleic acid of claim 5, which encodes a polypeptide having the amino acid sequence of the extracellular domain shown in Figure 1A-1B for AL-2.

7. The isolated nucleic acid of claim 1, wherein AL-2 is joined to an immunoglobulin .

8. The isolated nucleic acid of claim 7, which encodes AL-2-IgG.

9. The isolated nucleic acid of claim 1, wherein AL-2 is fused to a tag polypeptide.

10. The isolated nucleic acid of claim 1, which hybridizes to DNA encoding mature human AL-2l of Figure 1A-1B or mature human AL-2s of Figure 2A-2B under stringent conditions, and which encodes a polypeptide that is antigenically cross-reactive to mature human AL-2s or AL-2l.

11. An expression vector comprising the nucleotide sequence of claim 1 operably linked to a promoter.

12. The expression vector of claim 11, wherein the nucleotide sequence encodes the amino acid sequence for mature AL-2 shown in Figure 1A-1B or Figure 2A-2B.

13. The expression vector of claim 12, wherein the nucleotide sequence encoding the amino acid sequence for mature AL-2 is that shown in Figure 1A-1B or Figure 2A-2B.

14. A host cell transformed with the expression vector of claim 11.

15. The host cell of claim 14, wherein the nucleotide sequence encodes the amino acid sequence for mature AL-2 shown in Figure 1A-1B or Figure 2A-2B.

16. A method of using the host cell of claim 14, which method comprises culturing the host cell under conditions that allow replication of the expression vector.

17. A process which comprises transforming a host cell with an expression vector capable, in the host cell transformed with the vector, of expressing a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence shown in Figure 1A-1B or Figure 2A-2B for mature AL-2, and culturing the transformed host cell under conditions such that the AL-2 polypeptide is synthesized.

18. An isolated polypeptide having an amino acid sequence that is at least 75% homologous to the mature human AL-2 amino acid sequence shown in Figure 1A-1B or Figure 2A-2B.

37. The method of claim 36, wherein the angiogenesis-associated disease condition is rheumatoid arthritis or tumor formation.

38. A method of diagnosing a neurologic disease or disorder, comprising contacting nucleic acid of a sample with a second nucleic acid comprising at least 10 nucleotides of the nucleotide sequence shown in Figures 1A-1B or 2A-2B under conditions that allow hybridization of complementary nucleotide sequences, and detecting any hybridization that occurs.

39. The method of claim 38, further comprising amplifying the sample nucleic acid to which the second nucleic acid hybridizes.

```
   1  GNTCTAGAANTA GTGGATCCCCCC GGGCTGCAGGAA TTCCGACGGCCC CTGGAAGGGCTC TGGTGGGGCTGA
      CNAGATCTTNAT CACCTAGGGGGG CCCGACGTCCTT AAGGCTGCCGGG GACCTTCCCGAG ACCACCCCGACT

  73  GCGCTCTGCCGC GGGGGCGCGGGC ACAGCAGGAAGC AGGTCCGCGTGG GCGCTGGGGGCA TCAGCTACCGGG
      CGCGAGACGGCG CCCCCGCGCCCG TGTCGTCCTTCG TCCAGGCGCACC CGCGACCCCCGT AGTCGATGGCCC

 145  GTGGTCCGGGCT GAAGAGCCAGGC AGCCAAGGCAGC CACCCCGGGGGG TGGGCGACTTTG GGGGAGTTGGTG
      CACCAGGCCCGA CTTCTCGGTCCG TCGGTTCCGTCG GTGGGGCCCCCC ACCCGCTGAAAC CCCCTCAACCAC

 217  CCCCGCCCCCCA GGCCTTGGCGGG GTCATGGGGCCC CCCCATTCTGGG CCGGGGGGCGTG CGAGTCGGGGCC
      GGGGCGGGGGGT CCGGAACCGCCC CAGTACCCCGGG GGGGTAAGACCC GGCCCCCCGCAC GCTCAGCCCCGG
   1                                MetGlyPro ProHisSerGly ProGlyGlyVal ArgValGlyAla

 289  CTGCTGCTGCTG GGGGTTTTGGGG CTGGTGTCTGGG CTCAGCCTGGAG CCTGTCTACTGG AACTCGGCGAAT
      GACGACGACGAC CCCCAAAACCCC GACCACAGACCC GAGTCGGACCTC GGACAGATGACC TTGAGCCGCTTA
  16  LeuLeuLeuLeu GlyValLeuGly LeuValSerGly LeuSerLeuGlu ProValTyrTrp AsnSerAlaAsn

 361  AAGAGGTTCCAG GCAGAGGGTGGT TATGTGCTGTAC CCTCAGATCGGG GACCGGCTAGAC CTGCTCTGCCCC
      TTCTCCAAGGTC CGTCTCCCACCA ATACACGACATG GGAGTCTAGCCC CTGGCCGATCTG GACGAGACGGGG
  40  LysArgPheGln AlaGluGlyGly TyrValLeuTyr ProGlnIleGly AspArgLeuAsp LeuLeuCysPro

 433  CGGGCCCGGCCT CCTGGCCCTCAC TCCTCTCCTAAT TATGAGTTCTAC AAGCTGTACCTG GTAGGGGGTGCT
      GCCCGGGCCGGA GGACCGGGAGTG AGGAGAGGATTA ATACTCAAGATG TTCGACATGGAC CATCCCCCACGA
  64  ArgAlaArgPro ProGlyProHis SerSerProAsn TyrGluPheTyr LysLeuTyrLeu ValGlyGlyAla

 505  CAGGGCCGGCGC TGTGAGGCACCC CCTGCCCCAAAC CTCCTTCTCACT TGTGATCGCCCA GACCTGGATCTC
      GTCCCGGCCGCG ACACTCCGTGGG GGACGGGGTTTG GAGGAAGAGTGA ACACTAGCGGGT CTGGACCTAGAG
  88  GlnGlyArgArg CysGluAlaPro ProAlaProAsn LeuLeuLeuThr CysAspArgPro AspLeuAspLeu

 577  CGCTTCACCATC AAGTTCCAGGAG TATAGCCCTAAT CTCTGGGGCCAC GAGTTCCGCTCG CACCACGATTAC
      GCGAAGTGGTAG TTCAAGGTCCTC ATATCGGGATTA GAGACCCCGGTG CTCAAGGCGAGC GTGGTGCTAATG
 112  ArgPheThrIle LysPheGlnGlu TyrSerProAsn LeuTrpGlyHis GluPheArgSer HisHisAspTyr
```

FIG._1A

```
649  TACATCATTGCC ACATCGGATGGG ACCCGGGAGGGC CTGGAGAGCCTG CAGGGAGGTGTG TGCCTAACCAGA
     ATGTAGTAACGG TGTAGCCTACCC TGGGCCCTCCCG GACCTCTCGGAC GTCCCTCCACAC ACGGATTGGTCT
136  TyrIleIleAla ThrSerAspGly ThrArgGluGly LeuGluSerLeu GlnGlyGlyVal CysLeuThrArg

721  GGCATGAAGGTG CTTCTCCGAGTG GGACAAAGTCCC CGAGGAGGGGCT GTCCCCCGAAAA CCTGTGTCTGAA
     CCGTACTTCCAC GAAGAGGCTCAC CCTGTTTCAGGG GCTCCTCCCCGA CAGGGGGCTTTT GGACACAGACTT
160  GlyMetLysVal LeuLeuArgVal GlyGlnSerPro ArgGlyGlyAla ValProArgLys ProValSerGlu

793  ATGCCCATGGAA AGAGACCGAGGG GCAGCCCACAGC CTGGAGCCTGGG AAGGAGAACCTG CCAGGTGACCCC
     TACGGGTACCTT TCTCTGGCTCCC CGTCGGGTGTCG GACCTCGGACCC TTCCTCTTGGAC GGTCCACTGGGG
184  MetProMetGlu ArgAspArgGly AlaAlaHisSer LeuGluProGly LysGluAsnLeu ProGlyAspPro

865  ACCAGCAATGCA ACCTCCCGGGGT GCTGAAGGCCCC CTGCCCCCTCCC AGCATGCCTGCA GTGGCTGGGGCA
     TGGTCGTTACGT TGGAGGGCCCCA CGACTTCCGGGG GACGGGGGAGGG TCGTACGGACGT CACCGACCCCGT
208  ThrSerAsnAla ThrSerArgGly AlaGluGlyPro LeuProProPro SerMetProAla ValAlaGlyAla

937  GCAGGGGGGCTG GCGCTGCTCTTG CTGGGCGTGGCA GGGGCTGGGGGT GCCATGTGTTGG CGGAGACGGCGG
     CGTCCCCCCGAC CGCGACGAGAAC GACCCGCACCGT CCCCGACCCCCA CGGTACACAACC GCCTCTGCCGCC
232  AlaGlyGlyLeu AlaLeuLeuLeu LeuGlyValAla GlyAlaGlyGly AlaMetCysTrp ArgArgArgArg

1009 GCCAAGCCTTCG GAGAGTCGCCAC CCTGGTCCTGGC TCCTTCGGGAGG GGAGGGTCTCTG GGCCTGGGGGGT
     CGGTTCGGAAGC CTCTCAGCGGTG GGACCAGGACCG AGGAAGCCCTCC CCTCCCAGAGAC CCGGACCCCCCA
256  AlaLysProSer GluSerArgHis ProGlyProGly SerPheGlyArg GlyGlySerLeu GlyLeuGlyGly

1081 GGAGGTGGGATG GGACCTCGGGAG GCTGAGCCTGGG GAGCTAGGGATA GCTCTGCGGGGT GGCGGGGCTGCA
     CCTCCACCCTAC CCTGGAGCCCTC CGACTCGGACCC CTCGATCCCTAT CGAGACGCCCCA CCGCCCCGACGT
280  GlyGlyGlyMet GlyProArgGlu AlaGluProGly GluLeuGlyIle AlaLeuArgGly GlyGlyAlaAla

1153 GATCCCCCCTTC TGCCCCCACTAT GAGAAGGTGAGT GGTGACTATGGG CATCCTGTGTAT ATCGTGCAGGAT
     CTAGGGGGGAAG ACGGGGGTGATA CTCTTCCACTCA CCACTGATACCC GTAGGACACATA TAGCACGTCCTA
304  AspProProPhe CysProHisTyr GluLysValSer GlyAspTyrGly HisProValTyr IleValGlnAsp

1225 GGGCCCCCCCAG AGCCCTCCAAAC ATCTACTACACA TCGATTTCTGTG TTGGAGTGGCCC ATATTGCATACG
     CCCGGGGGGGGTC TCGGGAGGTTTG TAGATGATGTGT AGCTAAAGACAC AACCTCACCGGG TATAACGTATGC
328  GlyProProGln SerProProAsn IleTyrTyrThr SerIleServal LeuGluTrpPro IleLeuHisThr
```

FIG._1B

```
1297  ATACAACTGTTT TTCATGCGATCC AAGTGCTCCCGT GTCACTACATTC TTATTTCCTGTG CAAGTTATTACG
      TATGTTGACAAA AAGTACGCTAGG TTCACGAGGGCA CAGTGATGTAAG AATAAAGGACAC GTTCAATAATGC
 352  IleGlnLeuPhe PheMetArgSer LysCysSerArg ValThrThrPhe LeuPheProVal GlnValIleThr

1369  ACATCGACTTGC CGGATGACTTCA TTTAGCTTTACC ACCCTGAACCCA TCCATGCAGGCC TGCAGAGCACAG
      TGTAGCTGAACG GCCTACTGAAGT AAATCGAAATGG TGGGACTTGGGT AGGTACGTCCGG ACGTCTCGTGTC
 376  ThrSerThrCys ArgMetThrSer PheSerPheThr ThrLeuAsnPro SerMetGlnAla CysArgAlaGln

1441  ATGGGGGAATTC CGAATCAGATGG TGTTTCTGGGGG GACAGGATCCTG GGTACGGCTCTG TTTGTGCTTGTG
      TACCCCCTTAAG GCTTAGTCTACC ACAAAGACCCCC CTGTCCTAGGAC CCATGCCGAGAC AAACACGAACAC
 400  MetGlyGluPhe ArgIleArgTrp CysPheTrpGly AspArgIleLeu GlyThrAlaLeu PheValLeuVal

1513  CTTATTCTTCTT CTTGGGAGGCTG AATATGCATCAG ACGACACTGCTC CGGCAACGGGCC AGTGTGGAGGCG
      GAATAAGAAGAA GAACCCTCCGAC TTATACGTAGTC TGCTGTGACGAG GCCGTTGCCCGG TCACACCTCCGC
 424  LeuIleLeuLeu LeuGlyArgLeu AsnMetHisGln ThrThrLeuLeu ArgGlnArgAla SerValGluAla

1585  GAAGCCGGCCAG CATGGTCCCCTG TGATAGGATTGA AAGAGCTACTGA GAATAGGGGGCT TCTCAATGAGAG
      CTTCGGCCGGTC GTACCAGGGGAC ACTATCCTAACT TTCTCGATGACT CTTATCCCCCGA AGAGTTACTCTC
 448  GluAlaGlyGln HisGlyProLeu

1657  AGCGGAGGCTGC TGTTATCATGGG AACCAGGCAGAT CAATCATCCCTG GCAGGTCAGGCA GGAAGTTACTTA
      TCGCCTCCGACG ACAATAGTACCC TTGGTCCGTCTA GTTAGTAGGGAC CGTCCAGTCCGT CCTTCAATGAAT

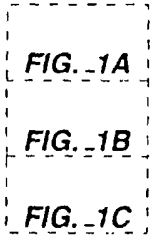
1729  GCTTCTCCTTCA CCTTCTTCCCAC AGAATTTATTAT AGGCTTGTTCCA AGTTGTAGTGTG TGATCAGATTCG
      CGAAGAGGAAGT GGAAGAAGGGTG TCTTAAATAATA TCCGAACAAGGT TCAACATCACAC ACTAGTCTAAGC

1801  TGCTGCCTGTCA GCTCTGTGCTAC CTGGCAGTTCCC CTCATGGAATTC GATATCAAGCTT ATCGATACCGTC
      ACGACGGACAGT CGAGACACGATG GACCGTCAAGGG GAGTACCTTAAG CTATAGTTCGAA TAGCTATGGCAG

1873  GACCT
      CTGGA
```

FIG._1C

FIG._1



```
   1  GNTCTAGAANTA GTGGATCCCCCC GGGCTGCAGGAA TTCCGACGGCCC CTGGAAGGGCTC TGGTGGGGCTGA
      CNAGATCTTNAT CACCTAGGGGGG CCCGACGTCCTT AAGGCTGCCGGG GACCTTCCCGAG ACCACCCCGACT

  73  GCGCTCTGCCGC GGGGGCGCGGGC ACAGCAGGAAGC AGGTCCGCGTGG GCGCTGGGGGCA TCAGCTACCGGG
      CGCGAGACGGCG CCCCCGCGCCCG TGTCGTCCTTCG TCCAGGCGCACC CGCGACCCCCGT AGTCGATGGCCC

 145  GTGGTCCGGGCT GAAGAGCCAGGC AGCCAAGGCAGC CACCCCGGGGGG TGGGCGACTTTG GGGGAGTTGGTG
      CACCAGGCCCGA CTTCTCGGTCCG TCGGTTCCGTCG GTGGGGCCCCCC ACCCGCTGAAAC CCCCTCAACCAC

 217  CCCCGCCCCCCA GGCCTTGGCGGG GTCATGGGGCCC CCCCATTCTGGG CCGGGGGGCGTG CGAGTCGGGGCC
      GGGGCGGGGGGT CCGGAACCGCCC CAGTACCCCGGG GGGGTAAGACCC GGCCCCCCGCAC GCTCAGCCCCGG
        1                       MetGlyPro  ProHisSerGly  ProGlyGlyVal  ArgValGlyAla

 289  CTGCTGCTGCTG GGGGTTTTGGGG CTGGTGTCTGGG CTCAGCCTGGAG CCTGTCTACTGG AACTCGGCGAAT
      GACGACGACGAC CCCCAAAACCCC GACCACAGACCC GAGTCGGACCTC GGACAGATGACC TTGAGCCGCTTA
  16  LeuLeuLeuLeu GlyValLeuGly LeuValSerGly LeuSerLeuGlu ProValTyrTrp AsnSerAlaAsn

 361  AAGAGGTTCCAG GCAGAGGGTGGT TATGTGCTGTAC CCTCAGATCGGG GACCGGCTAGAC CTGCTCTGCCCC
      TTCTCCAAGGTC CGTCTCCCACCA ATACACGACATG GGAGTCTAGCCC CTGGCCGATCTG GACGAGACGGGG
  40  LysArgPheGln AlaGluGlyGly TyrValLeuTyr ProGlnIleGly AspArgLeuAsp LeuLeuCysPro

 433  CGGGCCCGGCCT CCTGGCCCTCAC TCCTCTCCTAAT TATGAGTTCTAC AAGCTGTACCTG GTAGGGGGTGCT
      GCCCGGGCCGGA GGACCGGGAGTG AGGAGAGGATTA ATACTCAAGATG TTCGACATGGAC CATCCCCCACGA
  64  ArgAlaArgPro ProGlyProHis SerSerProAsn TyrGluPheTyr LysLeuTyrLeu ValGlyGlyAla

 505  CAGGGCCGGCGC TGTGAGGCACCC CCTGCCCCAAAC CTCCTTCTCACT TGTGATCGCCCA GACCTGGATCTC
      GTCCCGGCCGCG ACACTCCGTGGG GGACGGGGTTTG GAGGAAGAGTGA ACACTAGCGGGT CTGGACCTAGAG
  88  GlnGlyArgArg CysGluAlaPro ProAlaProAsn LeuLeuLeuThr CysAspArgPro AspLeuAspLeu

 577  CGCTTCACCATC AAGTTCCAGGAG TATAGCCCTAAT CTCTGGGGCCAC GAGTTCCGCTCG CACCACGATTAC
      GCGAAGTGGTAG TTCAAGGTCCTC ATATCGGGATTA GAGACCCCGGTG CTCAAGGCGAGC GTGGTGCTAATG
 112  ArgPheThrIle LysPheGlnGlu TyrSerProAsn LeuTrpGlyHis GluPheArgSer HisHisAspTyr
```

FIG._2A

```
649  TACATCATTGCC ACATCGGATGGG ACCCGGGAGGGC CTGGAGAGCCTG CAGGGAGGTGTG TGCCTAACCAGA
     ATGTAGTAACGG TGTAGCCTACCC TGGGCCCTCCCG GACCTCTCGGAC GTCCCTCCACAC ACGGATTGGTCT
136  TyrIleIleAla ThrSerAspGly ThrArgGluGly LeuGluSerLeu GlnGlyGlyVal CysLeuThrArg

721  GGCATGAAGGTG CTTCTCCGAGTG GGACAAAGTCCC CGAGGAGGGGCT GTCCCCCGAAAA CCTGTGTCTGAA
     CCGTACTTCCAC GAAGAGGCTCAC CCTGTTTCAGGG GCTCCTCCCCGA CAGGGGGCTTTT GGACACAGACTT
160  GlyMetLysVal LeuLeuArgVal GlyGlnSerPro ArgGlyGlyAla ValProArgLys ProValSerGlu

793  ATGCCCATGGAA AGAGACCGAGGG GCAGCCCACAGC CTGGAGCCTGGG AAGGAGAACCTG CCAGGTGACCCC
     TACGGGTACCTT TCTCTGGCTCCC CGTCGGGTGTCG GACCTCGGACCC TTCCTCTTGGAC GGTCCACTGGGG
184  MetProMetGlu ArgAspArgGly AlaAlaHisSer LeuGluProGly LysGluAsnLeu ProGlyAspPro

865  ACCAGCAATGCA ACCTCCCGGGGT GCTGAAGGCCCC CTGCCCCCTCCC AGCATGCCTGCA GTGGCTGGGGCA
     TGGTCGTTACGT TGGAGGGCCCCA CGACTTCCGGGG GACGGGGGAGGG TCGTACGGACGT CACCGACCCCGT
208  ThrSerAsnAla ThrSerArgGly AlaGluGlyPro LeuProProPro SerMetProAla ValAlaGlyAla

937  GCAGGGGGGCTG GCGCTGCTCTTG CTGGGCGTGGCA GGGGCTGGGGGT GCCATGTGTTGG CGGAGACGGCGG
     CGTCCCCCCGAC CGCGACGAGAAC GACCCGCACCGT CCCCGACCCCCA CGGTACACAACC GCCTCTGCCGCC
232  AlaGlyGlyLeu AlaLeuLeuLeu LeuGlyValAla GlyAlaGlyGly AlaMetCysTrp ArgArgArgArg

1009 GCCAAGCCTTCG GAGAGTCGCCAC CCTGGTCCTGGC TCCTTCGGGAGG GGAGGGTCTCTG GGCCTGGGGGGT
     CGGTTCGGAAGC CTCTCAGCGGTG GGACCAGGACCG AGGAAGCCCTCC CCTCCCAGAGAC CCGGACCCCCCA
256  AlaLysProSer GluSerArgHis ProGlyProGly SerPheGlyArg GlyGlySerLeu GlyLeuGlyGly

1081 GGAGGTGGGATG GGACCTCGGGAG GCTGAGCCTGGG GAGCTAGGGATA GCTCTGCGGGGT GGCGGGGCTGCA
     CCTCCACCCTAC CCTGGAGCCCTC CGACTCGGACCC CTCGATCCCTAT CGAGACGCCCCA CCGCCCCGACGT
280  GlyGlyGlyMet GlyProArgGlu AlaGluProGly GluLeuGlyIle AlaLeuArgGly GlyGlyAlaAla

1153 GATCCCCCCTTC TGCCCCCACTAT GAGAAGGTGAGT GGTGACTATGGG CATCCTGTGTAT ATCGTGCAGGAT
     CTAGGGGGGAAG ACGGGGGTGATA CTCTTCCACTCA CCACTGATACCC GTAGGACACATA TAGCACGTCCTA
304  AspProProPhe CysProHisTyr GluLysValSer GlyAspTyrGly HisProValTyr IleValGlnAsp

1225 GGGCCCCCCCAG AGCCCTCCAAAC ATCTACTACAAG GTATGAGGGCTC CTCTCACGTGGC TATCCTGAATCC
     CCCGGGGGGGTC TCGGGAGGTTTG TAGATGATGTTC CATACTCCCGAG GAGAGTGCACCG ATAGGACTTAGG
328  GlyProProGln SerProProAsn IleTyrTyrLys ValOP*
```

FIG._2B

```
1297  AGCCCTTCTTGG GGTGCTCCTCCA GTTTAATTCCTG GTTTGAGGGACA CCTCTAACATCT CGGCCCCCTGTG
      TCGGGAAGAACC CCACGAGGAGGT CAAATTAAGGAC CAAACTCCCTGT GGAGATTGTAGA GCCGGGGGACAC

1369  CCCCCCCAGCCC CTTCACTCCTCC CGGCTGCTGTCC TCGTCTCCACTT TTAGGATTCCTT AGGATTCCCACT
      GGGGGGGTCGGG GAAGTGAGGAGG GCCGACGACAGG AGCAGAGGTGAA AATCCTAAGGAA TCCTAAGGGTGA

1441  GCCCCACTTCCT GCCCTCCCGTTT GGCCATGGGTGC CCCCCTCTGTCT CAGTGTCCCTGG ATCCTTTTTCCT
      CGGGGTGAAGGA CGGGAGGGCAAA CCGGTACCCACG GGGGGAGACAGA GTCACAGGGACC TAGGAAAAAGGA

1513  TGGGGAGGGGCA CAGGCTCAGCCT CCTCTCTGACCA TGACCCAGGCAT CCTTGTCCCCCT CACCCACCCAGA
      ACCCCTCCCCGT GTCCGAGTCGGA GGAGAGACTGGT ACTGGGTCCGTA GGAACAGGGGGA GTGGGTGGGTCT

1585  GCTAGGGGCGGG AACAGCCCACCT TTTGGTTGGCAC CGCCTTCTTTCT GCCTCTCACTGG TTTTCTCTTCTC
      CGATCCCCGCCC TTGTCGGGTGGA AAACCAACCGTG GCGGAAGAAAGA CGGAGAGTGACC AAAAGAGAAGAG

1657  TATCTCTTATTC TTTCCCTCTCTT CCGTCTCTAGGT CTGTTCTTCTTC CCTAGCATCCTC CTCCCCACATCT
      ATAGAGAATAAG AAAGGGAGAGAA GGCAGAGATCCA GACAAGAAGAAG GGATCGTAGGAG GAGGGGTGTAGA

1729  CCTTTCACCCTC TTGGCTTCTTAT CCTGTGCCTCTC CCATCTCCTGGG TGGGGGCATCAA AGCATTTCTCCC
      GGAAAGTGGGAG AACCGAAGAATA GGACACGGAGAG GGTAGAGGACCC ACCCCCGTAGTT TCGTAAAGAGGG

1801  CTTAGCTTTCAG CCCCCCTTCTGA CCTCTCATACCA ACCACTCCCCTC AGTCTGCCAAAA ATGGGGGCCTTA
      GAATCGAAAGTC GGGGGGAAGACT GGAGAGTATGGT TGGTGAGGGGAG TCAGACGGTTTT TACCCCCGGAAT

1873  TGGGGAAGGCTC TGACACTCCACC CCAGCTCAGGCC ATGGGCACCAGG GCTCCATTCTCT GGCCTGGCCCAG
      ACCCCTTCCGAG ACTGTGAGGTGG GGTCGAGTCCGG TACCCGTCGTCC CGAGGTAAGAGA CCGGACCGGGTC

1945  GCCTCTACATAC TTACTCCAGCCA TTTGGGGTGGTT GGGTCATGACAG CTACCATGAGAA GAAGTGTCCCGT
      CGGAGATGTATG AATGAGGTCGGT AAACCCCACCAA CCCAGTACTGTC GATGGTACTCTT CTTCACAGGGCA

2017  TTTGTCCAGTGG CCAATAGCAAGA TATGAACCGGTC GGGACATGTATG GACTTGGTCTGA TGCTGAATGGGC
      AAACAGGTCACC GGTTATCGTTCT ATACTTGGCCAG CCCTGTACATAC CTGAACCAGACT ACGACTTACCCG
```

FIG._2C

```
2089  CACTTGGGACCG GAAGTGACTTGC TCCAGACAAGAG GTGACCAGGCCC GGACAGAAATGG CCTGGGAAGTAG
      GTGAACCCTGGC CTTCACTGAACG AGGTCTGTTCTC CACTGGTCCGGG CCTGTCTTTACC GGACCCTTCATC

2161  CAGAAGCAGTGC AGCAGGAACTGG AAGTGCCTTCAT CCAGGACAGGAA GTAGCACTTCTG AAACAGGAAGTG
      GTCTTCGTCACG TCGTCCTTGACC TTCACGGAAGTA GGTCCTGTCCTT CATCGTGAAGAC TTTGTCCTTCAC

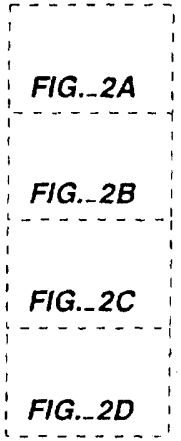
2233  GTCTGGCTGGAA CTCCAAGTGGCT TAGTCTGGGGGA TCAGGAGGTGGG AGGTGGATGGTT CTTATTCTGTGG
      CAGACCGACCTT GAGGTTCACCGA ATCAGACCCCCT AGTCCTCCACCC TCCACCTACCAA GAATAAGACACC

2305  AGAAGAAGGGCG GGAAGAACTTCC TTTCAGGAGGAA GCTGGAACTTAC TGACTGTAAGAG GTTAGAGGTGGA
      TCTTCTTCCCGC CCTTCTTGAAGG AAAGTCCTCCTT CGACCTTGAATG ACTGACATTCTC CAATCTCCACCT

2377  CCGA
      GGCT
```

FIG._2D

FIG._2



```
AL-2b.L    1 G N T C T A G A A N T A G T G G A T C C C C C C G G G C T G C A G G A A T T C C G A C G G C C C C T
AL-2b.L   51 G G A A G G G C T C T G G T G G G G C T G A G C G C T C T G C C G C G G G G G C G C G G G C A C A G
AL-2b.L  101 C A G G A A G C A G G T C C G C G T G G G C G C T G G G G G C A T C A G C T A C C G G G G G T G G T C
AL-2b.L  151 C G G G C T G A A G A G C C A G G C A G C C A A G G C A G C C A C C C C G G G G G G G T G G G C G A C
AL-2b.L  201 T T T G G G G G A G T T G G T G C C C C G C C C C C C C A G G C C T T G G C G G G G G T C A T G G G G C
AL-2b.L  251 C C C C C C C A T T C T G G G C C G G G G G G C G T G C G A G T C G G G G C C C C T G C T G C T G C T G
AL-2b.L  301 G G G G T T T T G G G G C T G G T G T C T G G G C T C A G C C C T G G A G C C C T G T C T A C T G G A A
AL-2b.L  351 C T C G G C G A A T A A G A G G T T C C A G G C A G A G G G T G G T T A T G T G C T G T A C C C T C
AL-2b.L  401 A G A T C G G G G A C C G G C T A G A C C T G C T C T G C C C C C G G G C C C G G C C T C C T G G C
AL-2b.L  451 C C T C A C T C C T C T C C T A A T T A T G A G T T C T A C A A G C T G T A C C T G G T A G G G G G
AL-2b.L  501 T G C T C A G G G C C C G G C G C T G T G A G G C A C C C C C T G C C C C A A A C C T C C T T C T C A
H10006     1 - - - - - - - - - G C C C G A C G C T G T G A G G C A C C C C C T G C C C C A A A C C T C C T T C T C A
AL-2b.L  551 C T T G T G A T C G C C C A G A C C T G G A T C T C C G C T T C A C C A T C A A G T T C C A G G A G
H10006    43 C T T G T G A T C G C C C A G A C C T G G A T C T C C G C T T C A C C A T C A A G T T C C A G G A G
AL-2b.L  601 T A T A G C C C T A A T C T C T G G G G C C A C G A G T T C C G C T C G C A C C A C G A T T A C T A
H10006    93 T A T A G C C C T A A T C T C T G G G G C C A C G A G T T C C G C T C G C A C C A C G A T T A C T A
AL-2b.L  651 C A T C A T T G C C A C A T C G G A T G G G A C C C G G G A G G G C C T G G A G A G C C T G C A G G
H10006   143 C A T C A T T G C C A C A T C G G A T G G G A C C C G G G A G G C C T G G G A G A G C C T G C A G G
AL-2b.L  701 G A G G T G T G T G C C T A A C C A G A G G C A T G A A G G T G C T T C T C C G A G T G G G A C A A
H10006   193 G A A G T G T G T G C C T A A C C A G A G G C A T G A A G G T G C T T C T C C G A G T N G G A C A A
```

FIG._3A

```
AL-2b.L   751 A G T C C C C G A G G A G G G G C T G T C C C C C G A A A A C C T G T G T C T G A A A T G C C C A T
H10006    243 A G T - C C C G A G G A G G G G C T G T C C C C C G A A A A C C T G T G T C T G A A A T G C C C A T

AL-2b.L   801 G G A A A G A G A C C G A G G G G C A G C C C A C A G C C T - G G A G C C T - G G G A A G G A G A A
H10006    292 G G A A A G A G A C C G A G G G G C A G C C C A C A G C C T G G G A G C C T G G G G A A G G A G A A

AL-2b.L   849 C C T G C C A G G T G A C C C C A C C A G C A A T G C A A C C T C C C G G G G T - - G C T G A A G G
H10006    342 C C T G C C A G G T G A C C C C A C C A G C A A T N C A A C C T T C C G G G G T T G C T T G A A G G

AL-2b.L   897 C C C C C T - - G C C C C C T C C C A G C A - T G C C T G C A G T - - G G C T G G G G C A G C A G G
H10006    392 G C C C C T T G A C C C T T T C C C A G C A T T G C N T G C A N T T G G T T N G G G G C A G C A N G

AL-2b.L   942 G G G G C - - - - T G G C G C T G C T C T T G C T G G G C G T G G C A G G G G C T G G G G G T G C C
H10006    442 G G G G N G T T T T G G C

AL-2b.L   988 A T G T G T T G G C G G A G A C G G C G G G C C A A G C C T T C G G A G A G T C G C C A C C C T G G

AL-2b.L  1038 T C C T G G C T C C T T C G G G A G G G G A G G G T C T C T G G G C C T G G G G G G T G G A G G T G

AL-2b.L  1088 G G A T G G G A C C T C G G G A G G C T G A G C C T G G G G A G C T A G G G A T A G C T C T G C G G

AL-2b.L  1138 G G T G G C G G G G C T G C A G A T C C C C C C T T C T G C C C C C A C T A T G A G A A G G T G A G

AL-2b.L  1188 T G G T G A C T A T G G G C A T C C T G T G T A T A T C G T G C A G G A T G G G C C C C C C C A G A

AL-2b.L  1238 G C C C T C C A A A C A T C T A C T A C A C A T C G A T T T C T G T G T T G G A G T G G C C C A T A

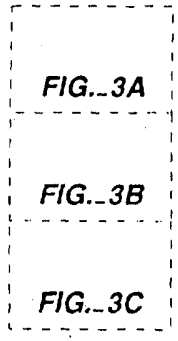
AL-2b.L  1288 T T G C A T A C G A T A C A A C T G T T T T T C A T G C G A T C C A A G T G C T C C C G T G T C A C
```

FIG._3B

```
AL-2b.L 1338 T A C A T T C T T A T T T C C T G T G C A A G T T A T T A C G A C A T C G A C T T G C C G G A T G A
AL-2b.L 1388 C T T C A T T T A G C T T T A C G A C C C T G A A C C C A T C C A T G C A G G C C T G C A G A G C A
AL-2b.L 1438 C A G A T G G G G G A A T T C C G A A T C A G A T G G T G T T T C T G G G G G G A C A G G A T C C T
AL-2b.L 1488 G G G T A C G G C T C T G T T T G T G C T T G T G C T T A T T C T T C T T C T T G G G A G G C T G A
AL-2b.L 1538 A T A T G C A T C A G A C G A C A G T G C T C C G G C A A C G G G C C A G T G T G G A G G C G G A A
AL-2b.L 1588 G C C G G C C A G C A T G G T C C G C T G T G A T A G G A T T G A A A G A G C T A C T G A G A A T A
AL-2b.L 1638 G G G G G C T T C T C A A T G A G A G A G C G G A G G C T G C T G T T A T C A T G G G A A C C A G G
AL-2b.L 1688 C A G A T C A A T C A T C C C T G G C A G G T C A G G C A G G A A G T T A C T T A G C T T C T C C T
AL-2b.L 1738 T C A C C T T C T T C C C A C A G A A T T T A T T A T A G G C T T G T T C C A A G T T G T A G T G T
AL-2b.L 1788 G T G A T C A G A T T C G T G C T G C C T G T C A G C T C T G T G C T A C C T G G C A G T T C C C C
AL-2b.L 1838 T C A T G G A A T T C G A T A T C A A G C T T A T C G A T A C C G T C G A C C T
```

FIG._3C

FIG._3



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lerk2      1 MA-RPGQRWLGKWLVAMVVWALCRLATPLAKNLEPVSWSSLNPKFLSGKG
huHTKL     1 MAVRRDSVWKYCWGVLMV---LCRTAISKSIVLEPIYWNSSNSKFLPGQG
AL2.sht    1 MG-PPHSGPGGVRVGALLLLGVLGLVSGL--SLEPVYWNSANKRFQAEGG
AL2.long   1 MG-PPHSGPGGVRVGALLLLGVLGLVSGL--SLEPVYWNSANKRFQAEGG

lerk2     50 LVIYPKIGDKLDIICPRA---EAGR--PYEYYKLYLVRPEQAAACSTVLD
huHTKL    48 LVLYPQIGDKLDIICPKV---DSKTVGQYEYYKVYMVDKDQADRCTIKKE
AL2.sht   48 YVLYPQIGDRLDLLCPRARPPGPHSSPNYEFYKLYLVGGAQGRRCEAPPA
AL2.long  48 YVLYPQIGDRLDLLCPRARPPGPHSSPNYEFYKLYLVGGAQGRRCEAPPA

lerk2     95 PNVLVTCNRPEQEIRFTIKFQEFSPNYMGLEFKKHHDYYITSTSNGSLEG
huHTKL    95 NTPLLNCAKPDQDIKFTIKFQEFSPNLWGLEFQKNKDYYIISTSNGSLEG
AL2.sht   98 PNLLLTCDRPDLDLRFTIKFQEYSPNLWGHEFRSHHDYYIIATSDGTREG
AL2.long  98 PNLLLTCDRPDLDLRFTIKFQEYSPNLWGHEFRSHHDYYIIATSDGTREG

lerk2    145 LENREGGVCRTRTMKIIMKVGQDPNAVTPEQLTTSRPSKEADNTVKMATQ
huHTKL   145 LDNQEGGVCQTRAMKILMKVGQDASS-----AGSTRNKDPTRRPELEAG
AL2.sht  148 LESLQGGVCLTRGMKVLLRVGQSPRGGAVPRKPVSEMPMERDRGAAHSLE
AL2.long 148 LESLQGGVCLTRGMKVLLRVGQSPRGGAVPRKPVSEMPMERDRGAAHSLE
```

FIG._4A

```
lerk2      195 A P G S R G S L G D S D G K H E T V N Q E E K S G P G A S G G S S G D P D G F F N S K V A L F A A V
huHTKL     189 T N G - R S S T T S P F V K P N P G S S T D G N S A G H S G - - - - - - N N I L G S E V A L F A G I
AL2.sht    198 - P G K E N L P G D P T S N A T S R G A E G P L P P P S M P A V A G A A G G L - - - A L L L L G V A
AL2.long   198 - P G K E N L P G D P T S N A T S R G A E G P L P P P S M P A V A G A A G G L - - - A L L L L G V A

lerk2      245 G A G C V I F L L I I I F L T V L L L K L R K R H R K H T Q - Q R A A A L S L S T L A S P K G G S G
huHTKL     232 A S G C I I F I V I I I T L V V L L L K Y R R R H R K H S P - Q H T T T L S L S T L A T P K R S G N
AL2.sht    244 G A G G A - - - - - M C W R R R R A K P S E S R H P G P G S F G R G G S L G L G G - - - - G G G M G
AL2.long   244 G A G G A - - - - - M C W R R R R A K P S E S R H P G P G S F G R G G S L G L G G - - - - G G G M G

lerk2      294 T A G T E P S D I I I P L R - - - T T E N N Y C P H Y E K V S G D Y G H P V Y I V Q E M P P Q S P A
huHTKL     281 N N G S E P S D I I I P L R - - - T A D S V F C P H Y E K V S G D Y G H P V Y I V Q E M P P Q S P A
AL2.sht    285 P R E A E P G E L G I A L R G G G A A D P P F C P H Y E K V S G D Y G H P V Y I V Q D G P P Q S P P
AL2.long   285 P R E A E P G E L G I A L R G G G A A D P P F C P H Y E K V S G D Y G H P V Y I V Q D G P P Q S P P

lerk2      341 N I Y Y - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
huHTKL     328 N I Y Y - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
AL2.sht    335 N I Y Y - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
AL2.long   335 N I Y Y T S I S V L E W P I L H T I Q L F F M R S K C S R V T T F L F P V Q V I T T S T C R M T S F
```

FIG._4B

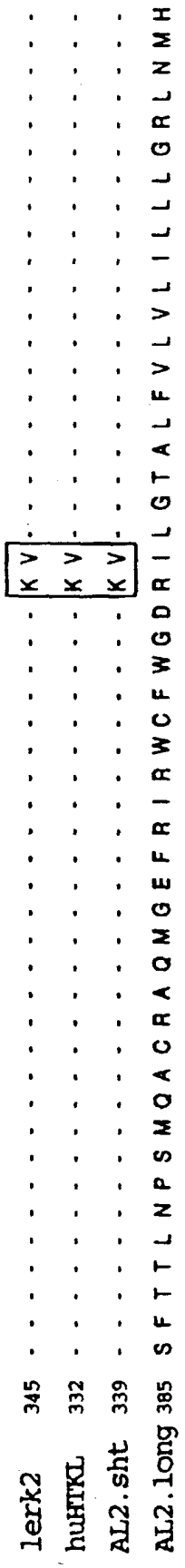

lerk2 345 . . . . . K V . . . . . H
huHTKL 332 . . . . . K V . . . . . H
AL2.sht 339 . . . . . K V . . . . . H
AL2.long 385 S F T T L N P S M Q A C R A Q M G E F R I R W C F W G D R I L G T A L F V L V L I L L L G R L N M H
AL2.long 435 Q T T L L R Q R A S V E A E A G Q H G P L

FIG._4C

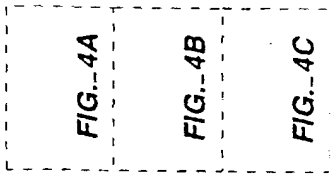


FIG._4A
FIG._4B
FIG._4C

FIG._4

```
LERK2      1 MA-RPGQRWLSKWLVAMVVLTLCRLATPLAKNLEPVSWSSLNPKFLSGKG
huHTKL     1 MAVRRDSVWKYCWGVLMV---LCRTAISKSIVLEPIYWNSSNSKFLPGQG
AL2.long   1 MGPPHSGP-GGVRVGALLL--LGVLGLVSGLSLEPVYWNSANKRFQAEGG

LERK2     50 LVIYPKIGDKLDIICPRA---EAGR--PYEYYKLYLVRPEQAAACSTVLD
huHTKL    48 LVLYPQIGDKLDIICPKV---DSKTVGQYEYYKVYMVDKDQADRCTIKKE
AL2.long  48 YVLYPQIGDRLDLLCPRARPPGPHSSPNYEFYKLYLVGGAQGRRCEAPPA

LERK2     95 PNVLVTCNKPHQEIRFTIKFQEFSPNYMGLEFKKYHDYYITSTSNGSLEG
huHTKL    95 NTPLLNCAKPDQDIKFTIKFQEFSPNLWGLEFQKNKDYYIISTSNGSLEG
AL2.long  98 PNLLLTCDRPDLDLRFTIKFQEYSPNLWGHEFRSHHDYYIIATSDGTREG

LERK2    145 LENREGGVCRTRTMKIVMKVGQDPNAVTPEQLTTSRPSKESDNTVKTATQ
huHTKL   145 LDNQEGGVCQTRAMKILMKVGQDASS------AGSTRNKDPTRRPELEAG
AL2.long 148 LESLQGGVCLTRGMKVLLRVGQSPRG------GAVPRKPVSEMPMERDRG

LERK2    195 APGRGSQGDSDGKHETVNQEEKSGPGAGGGGSGDSDSFFNSKVALFAAVG
huHTKL   189 TNGRSSTTSPFVKPNPGSSTDGNSAGHSG------NNILGSEVALFAGIA
AL2.long 192 AAHSLEPGKENLPGDPTSNATSRGAE---------GPLPPPSMPAVAGAA
```

FIG._5A

```
LERK2     245 AGCVIFLLIIIFLTVLLLKLRKRHRKHTQQRAAALSL---STLASPKGGS
huHTKL    233 SGCIIFIVIIITLVVLLLKYRRRHRKHSPQHTTTLSL---STLATPKRSG
AL2.long  233 GGLALLLLGVAGAGGAMCWRRRRAKPSESRHPGPGSFGRGGSLGLGGGGG

LERK2     292 -GTAGTEPSDIIIPLR---TTENNYCPHYEKVSGDYGHPVYIVQEMPPQS
huHTKL    280 -NNNGSEPSDIIIPLR---TADSVFCPHYEKVSGDYGHPVYIVQEMPPQS
AL2.long  283 MGPREAEPGELGIALRGGGAADPPFCPHYEKVSGDYGHPVYIVQDGPPQS

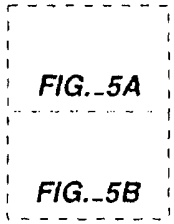
LERK2     338 PANIYY----------------------------------------------
huHTKL    326 PANIYY----------------------------------------------
AL2.long  333 PPNIYYTSISVLEWPILHTIQLFFMRSKCSRVTTFLFPVQVITTSTCRMT

LERK2     344 ------------------------------KV--------------------
huHTKL    332 ------------------------------KV--------------------
AL2.long  383 SFSFTTLNPSMQACRAQMGEFRIRWCFWGDRILGTALFVLVLILLLGRLN

AL2.long  433 MHQTTLLRQRASVEAEAGQHGPL
```

FIG._5B

FIG._5



# INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/06345

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/475 C12N15/62 A61K38/18 C07K16/22
C07K19/00 C12Q1/68 G01N33/50 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | NEURON, vol. 14, no. 5, 1995, pages 973-981, XP002038221 WINSLOW J.W. ET AL.: "Cloning of AL-1, a ligand for EpH-related tyrosine kinase receptor involved in axon bundle formation." cited in the application see the whole document, especially Fig. 4 --- | 1-17,38, 39 |
| Y | EMBL Database entry HS006163 Accession number H10006 02.07.1995 Hillier L. et al.: 'The WashU-Merck EST project.' XP002038224 cited in the application see abstract --- -/-- | 1-17,38, 39 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
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| 22 August 1997 | 05. 09. 97 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016 | Mandl, B |

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# INTERNATIONAL SEARCH REPORT

..formation on patent family members

Inter nal Application No
PCT/US 97/06345

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9527060 A | 12-10-95 | AU 2278995 A | 23-10-95 |
| | | CA 2187167 A | 12-10-95 |
| | | EP 0758381 A | 19-02-97 |
| | | ZA 9502762 A | 20-02-96 |
| WO 9715667 A | 01-05-97 | AU 7477896 A | 15-05-97 |